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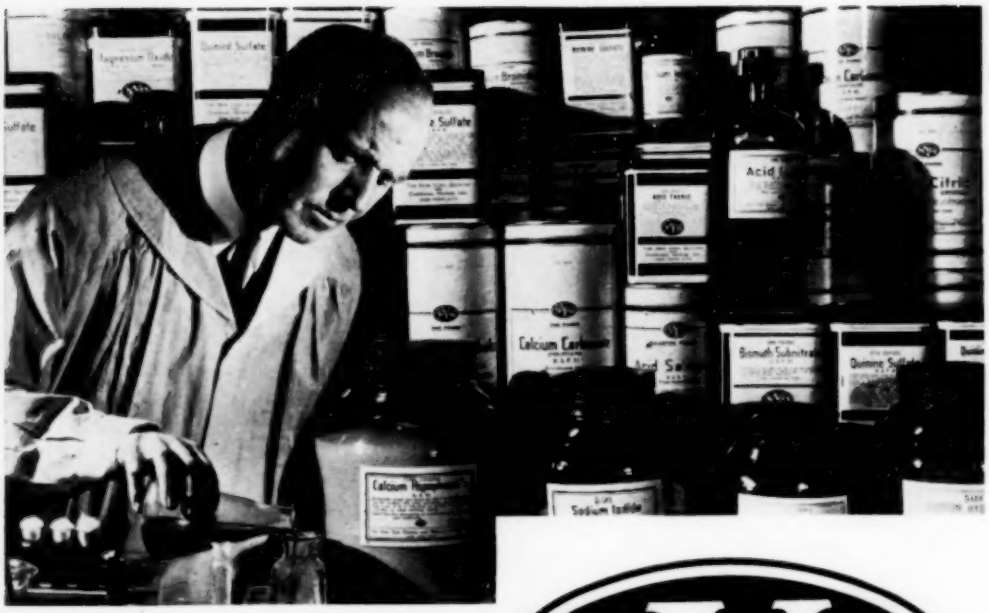

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JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

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A Pharmacognostical Study of European and American Arnicas*

By Heber W. Youngken† and Elmer H. Wirth‡

When our supply of Arnica flowers from *Arnica montana* was cut off by the war, we were forced to turn to our native species of *Arnica* as sources of the drug. Since about thirty recognized species of this genus occur in western North America, especially in the Rocky Mountain States, the problem of the selection of the proper American species presented itself. As little is known concerning the active constituents of Arnica other than the stimulating oleoresin contained in its glandular hairs, the choice of American Arnicas which might be considered desirable as additional sources of Arnica Flowers in the National Formulary was based upon (*a*) those whose flower heads possessed a sufficient abundance of glandular hairs and (*b*) the more abundantly occurring American species from which the drug might be obtainable commercially in sufficient amounts to meet the domestic demands.

The American species chosen for discussion in this paper, along with the European *Arnica montana*, are *Arnica fulgens* Pursh, *Arnica sororia* Greene and *Arnica cordifolia* Hooker. The flower heads of all of these are in current use as Arnica in this country and no report of inferiority of any of them to *Arnica montana* has come to our knowledge.

EXPERIMENTAL

Materials and Methods.—The materials used in this investigation consisted of herbarium sheets of *Arnica montana*, *A. fulgens*, *A. sororia*, *A. cordifolia* and other species of *Arnica* in the Gray Herbarium, herbaria of the Field Museum and of the Massachusetts College of Pharmacy, dried flower heads of *Arnica montana* from the collections of the College of Pharmacy of the University of Illinois and the Massachusetts College of Pharmacy, specimens of the flowering plants of *A. fulgens*, *A. sororia* and *A. cordifolia* furnished by Professor C. E. Mollett of the State University of Montana, and fresh and dried flower heads and entire plants collected by E. C. Moran of Stanford, Mont., and Richard V. Bausher of Allentown, Pa.

Herbarium specimens which were checked for authenticity by Professor Bassett Maguire, the outstanding American authority on the genus *Arnica*, furnished the basic materials used by us in determining the origin of the dried flower heads from herbarium sheets and from samples of dried commercial Arnica Flowers which were used in these studies.

Dried flower heads were boiled in water and dissected on the stage of a binocular dissecting micro-

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scope and studied. Macerated flower heads were also dissected in Syracuse watch glasses, their parts transferred to microslides, separately mounted in water, diluted glycerin and cyanin solution and studied with the aid of the compound microscope. Sections of the achenes were similarly mounted and examined. Measurements of the plant materials were made with a metric rule and the standardized ocular micrometer.

PLANTS

The genus *Arnica* is represented by 32 recognized species of perennial herbs distributed in the northerly and mountainous regions of the eastern and western hemispheres. Maguire (1) divides the genus into five subgenera, namely *Artica*, *Austromontana*, *Chamissonis*, *Montana* and *Andropurpurea*. In his classification, *Arnica montana* belongs to the subgenus *Montana*, *A. fulgens* and *A. sororia* to the subgenus *Artica*, and *A. cordifolia* to the subgenus *Austromontana*.

Arnica montana L., the principal source of European Arnica, commonly known as Mountain Arnica, Wolf's Bane and Mountain Tobacco, is a perennial herb native to the mountains and meadows of Europe. Its underground portion consists of a rhizome of horizontal growth up to 10 cm. in length and up to 5 mm. in diameter, showing in its anterior region annulations from the scars of previous year's leaves and remnants of last year's leaves and on its lower surface many coarse wiry rootlets. Its thick bark contains an interrupted circle of schizogenous oleoresin reservoirs. It was officially recognized in the U. S. P. of 1890 and is a richer source of volatile oil than the flowers, yielding, according to Wasicky (2), from 0.5 to 1.5 per cent of volatile oil on contrast to 0.04 to 0.14 per cent yields reported by the same author for Arnica Flowers.

Its overground portion consists of a basal rosette of entire oblanceolate leaves up to 17 cm. in length with 5 to 7 nerves from the center of which projects an erect, simple, glandular hairy stem up to 6 dm. in height which bears from 2 to 4 pairs of cauline leaves of ovate, elliptic-oblong, lanceolate or oblanceolate outline with rounded or rounded-toothed apex and clothed with numerous nonglandular and glandular hairs, up to 16 cm. in length in the material examined and up to 5 cm. in width. From the uppermost pair of cauline leaves extend 1 to 3 peduncles bearing alternate bracteoles. These are glandular-puberulent, each terminating in a hemispherical or turbinate capitulum up to 6 or 8 cm. in breadth bearing orange-yellow flowers. The fruit is a spindle-shaped achene from 5 to 7 mm. in length.

Hegi (3) gives the distribution of this species in northern Europe as northern France, Belgium, N. W. Germany, Denmark, Scandanavia, East and West Prussia, Pomerania, N. and E. Poland, Lithuania and Livonia; in southern Europe as Portugal, E. and N. Spain, to the Pyrenees to northern Italy, north Balkans and southern Russia.

Arnica fulgens Pursh, commonly known as Orange Arnica (4) possesses a horizontal rhizome which is, as reported by Maguire (5), thickly clothed with the bases of previous leaves in the axils of which occur dense tufts of long tawny hairs. Its radical leaves, occurring in a rosette, are lanceolate, petiolate and 3-nerved. Its puberulent, glandular-hairy stem arises to the height of 7 dm. and bears from 4 to 6 pairs of cauline leaves, the upper pairs reduced and usually separated by a long internode from the lower pairs, the latter oblanceolate, 4 to 5 nerved, entire or occasionally with a few teeth, the surface puberulent and glandular-hairy, the petiole winged. The peduncles (1 to 3) are up to 20 cm. in length, puberulent, glandular hairy, each terminating in a broad, hemispherical capitulum of dark orange to orange-yellow flowers. The fruit is a glandular hairy, oblong to fusiform achene from 4.5 to 5.5 mm. in length.

Maguire (5) gives its distribution and habitat as British Columbia, Alberta and Saskatchewan south to Oregon, northern Nevada, northern Utah and central Colorado occurring in the hills and prairies in the northern part of its range but toward the southern part climbing into the mountains to an altitude of 10,000 ft. or more. It is now being cultivated as a drug crop.

Arnica sororia Greene, another American species, is a perennial herb closely related to *A. fulgens* and sometimes mistaken for the latter. Its underground portion consists of a horizontal branched rhizome attaining less than half the thickness of that of *A. fulgens* and differing further from the latter by showing few or no tufts of hairs in the axils of old leaves. Numerous slender rootlets emanate from the lower surface of the rhizome and each of the branches or secondary rhizomes give rise to an aerial stem. Its slender, striate, villose, glandular-hairy stem arises to the height of 5 or 6 dm. and bears 4 or 5 pairs of cauline leaves, the 2 or 3 pairs near the base being oblanceolate, 3 to 5 nerved, entire or remotely toothed, petiolate, the petioles narrowly winged and as long as or somewhat shorter than the lamina, the upper two pairs of leaves sessile and reduced. The lower cauline leaves attain a length of about 14 cm. and a width of 2.5 cm. All of the leaves are puberulent and glandular-hairy. The slender peduncles vary from 5 to 15 cm. in length and are villose and glandular-pubescent. The flower heads possess yellow ray and disk florets subtended by an involucre of 2 series of narrowly lanceolate bracts. The hirsute achenes are mostly 3.5 to 5 mm. in length.

Maguire (5) gives its geographical distribution and habitat as Alberta and British Columbia south to north and western Wyoming, northern Utah and northern California, occurring on grassy and open slopes and hillsides.

Arnica cordifolia Hooker or Heart-leaf Arnica, another American species, is quite widespread and common in the Rocky Mountains. From a much-branched slender rhizome up to about 3 mm. in diameter arise slender, simple, villose, glandular pubescent stems often close together and attaining a length of 4.5 dm., rarely up to 6 dm. The cauline leaves occur in from 2 to 4 pairs, the basal and lower leaves being petiolate with slender, sometimes margined petioles as long as or exceeding the laminae, the latter varying from ovate to ovate-orbicular lanceolate or cordate, up to 10 cm. in length and up to 8 cm. in width, the apex varying from acute to obtuse and rounded, the base cordate, subcordate, truncate, or rarely acute, the margin entire to coarsely dentate. The upper leaves are sessile, ovate to lanceolate and considerably smaller than the lower ones. All of the leaves are puberulent and glandular-hairy. The slender, pilose and glandular-hairy peduncles are from 5 to 15 cm. in length and terminate in broad turbinate or campanulate capitula bearing yellow ray and disk florets. The number of heads per stem is usually 1 to 3 but occasionally up to 7 have been noted (5). The achenes are hirsute oblong to slender fusiform and mostly up to 6 mm. in length.

Maguire (5) has recorded its distribution from Alaska down the Rocky Mountains to New Mexico and Arizona, in the Sierra and Cascade ranges south to the Cuyamaca Mountains in San Diego Co., Calif., and in a restricted area in northern Michigan. Britton and Brown (6) also note its occurrence from Lake Superior to North Dakota and state it is recorded from western Nebraska.

GROSS STRUCTURE AND HISTOLOGY OF THE FLOWER HEADS

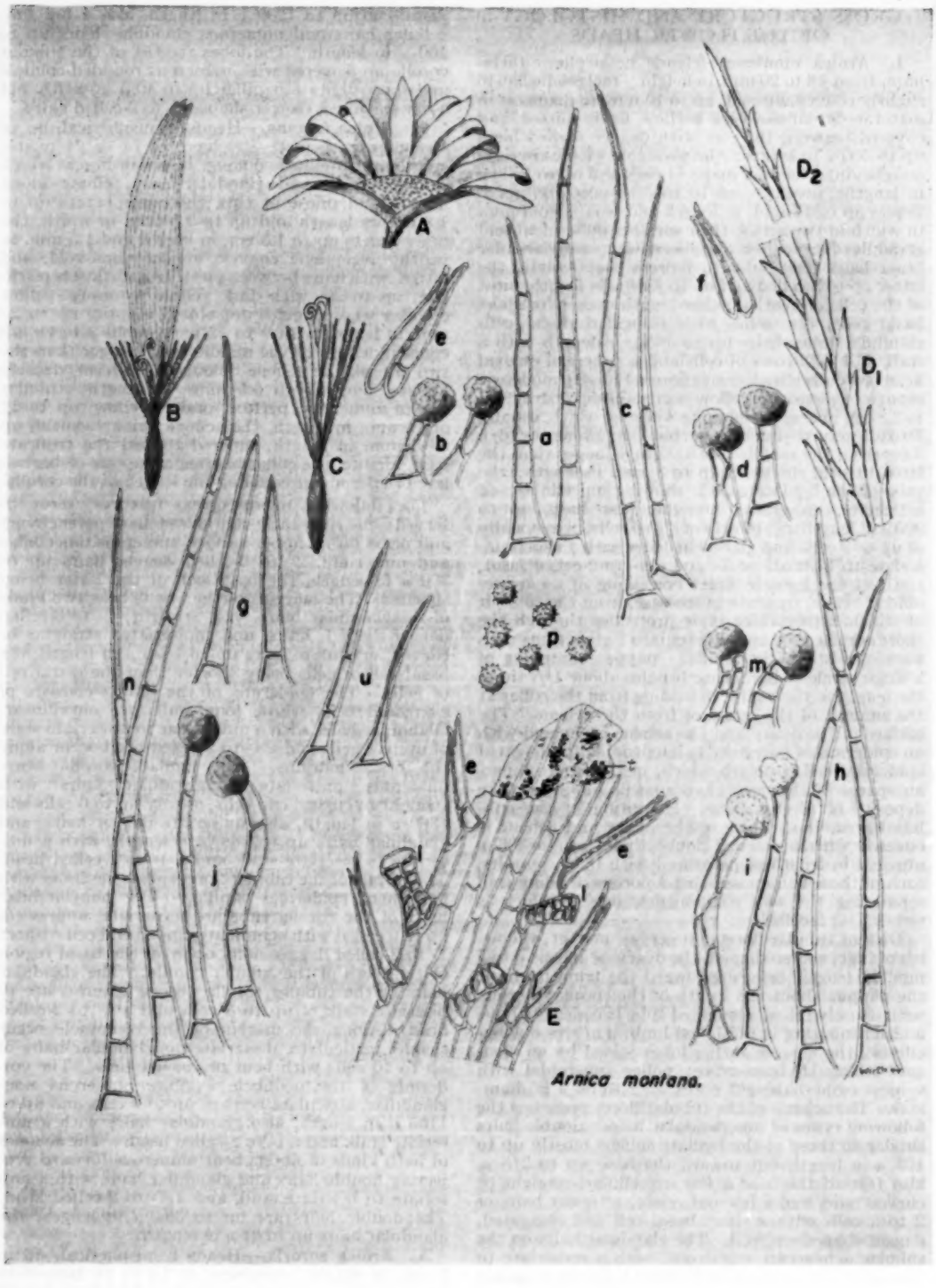
1. Arnica montana.—Heads hemispheric-turbinate, from 18 to 20 mm. in height; receptacle flat to slightly convex, discoid, up to 10 mm. in diameter in macerated material, its surface finely pitted and covered between the pits with dagger-shaped hairs up to 500 μ in length; the peduncle with uniseriate nonglandular hairs of up to 11 cells and up to 1530 μ in length; involucre of 16 to 32 lanceolate, acute bracts up to 17 mm. in length and from 1 to 3 mm. in width in two series, their surfaces puberulent and glandular-hairy, their margins ciliate; nonglandular bract hairs unicellular to uniseriate-articulate, the latter up to 8-celled and up to 1350 μ in length, most of the cells of nearly similar length except for short basal cells, but some with elongated distal cell; glandular bract hairs up to 595 μ in length with a stalk of 1 or 2 rows of cells and a spherical or oval head of 1 or 2 cells; ligulate florets 11 to 20, moderate orange to moderate yellow-orange in dried drug, up to 2.8 cm. in length and up to 3 mm. in width, usually 7 to 12 nerved [Fuchs reported 7 to 15 nerves (6)], 3-toothed, the middle tooth slightly longer than the lateral teeth, the teeth up to 3 mm. in length; the tube of the ligulate corolla showing unicellular and articulate, uniseriate, non-glandular hairs up to 900 μ in length and mostly of 3 to 6 cells, occasionally of up to 9 cells and glandular hairs with a short uniseriate to biseriate stalk and a 1- to 2-celled head; pistil of the ligulate floret consisting of an ovate-oblong ovary, truncate at its base, from the summit of which a threadlike style protrudes through the short corolla tube and divides into 2 style arms with numerous stigmatic papillae; pappus consisting of a single circle of barbellate bristles about 1¼ times the length of the ovary, extending from the collar at the summit of the ovary or from the achene. The surface of the ovary and the achene is covered with an epidermis of polygonal to longitudinally elongated epidermal cells beneath which, in the intercellular-air-spaces of the parenchyma occur broken netlike deposits of phytomelane. Excepting in the subbasal area, the surface of the ovary and achene is covered with numerous double hairs up to 300 μ unequal in length of parts and with bifid summits, each of these hairs possessing a porous dividing wall separating the two components, one of which is either 1- or 2-celled.

Disk or tubular florets numerous, perfect, yellowish orange, goblet-shaped, the ovary or achene 5 to 7 mm. in length, tapering toward the truncate base, the pappus about the length of the corolla; corolla with densely pilose, elongated tube broadened above and terminating in a 5-lobed limb; anthers oblong-elliptic, the narrow anther lobes joined by an elongate triangular connective; pollen spheroidal with spinose exine having 3 pores, 25.2 to 36 μ in diameter. The achene of the tubular floret possesses the following types of nonglandular hairs: double hairs similar to those of the ligulate achene mostly up to 190 μ in length but toward the base up to 218 μ, also toward the base a few unicellular, straight or curved hairs and a few uniseriate, articular hairs of 2 to 4 cells with a short basal cell and elongated, dagger-shaped end cell. The glandular hairs on the tubular achene are numerous, with a uniseriate to biseriate stalk and a 1- to 2-celled head, up to about 100 μ in length. As in the ligulate floret, the pappus consists of a single circle of barbellate bristles, each bristle possessing a multicellular axis bearing rows of forward projecting, unicellular pointed hairs with decurrent bases. Streaks of phytomelane occur in the intercellular spaces of the parenchyma beneath the epidermis of the achene. The tube of the corolla bears numerous uniseriate, articulate, nonglandular hairs with 1 to 4 short basal cells and an elongated distal cell up to 1200 μ in length, also a few unicellular hairs and numerous glandular hairs up to 100 μ in length. The lobes (teeth) of the tubular corolla are covered with numerous rounded-coinical and short digitate papillae up to 40 μ in width and a few scattered, dagger-shaped 1- to 2-celled hairs.

2. Arnica fulgens.—Heads hemispherical, up to 18.5 mm. in height; involucre of 14 to 24 ovate-lanceolate to elliptic-oblong, biseriate bracts which are pubescent and glandular-hairy, ciliate along margin and pilose at tips, the outer bracts up to 1.5 cm. in length and up to 3.5 mm. in width, the inner bracts up to 1.3 cm. in length and 1.5 mm. in width; receptacle convex, almost semiovoid, and pitted, with hairs between pits; ligulate florets pistillate, up to 20, with dark yellow to orange-yellow corollas whose ligules are oblong-elliptic, up to 25 mm. in length with 9 to 12 nerves and 3 teeth, all equal in length or the middle tooth longer than the two lateral, the achene oblong to fusiform, densely hirsute, and 4.5 to 5.5 mm. in length; tubular florets numerous, perfect, orange-yellow, up to 12 or 16 mm. in length, the achene oblong-conical, up to 5 mm. in length, tapered toward the truncate base, hirsute, the collar bearing a pappus of barbellate bristles about equaling the length of the corolla.

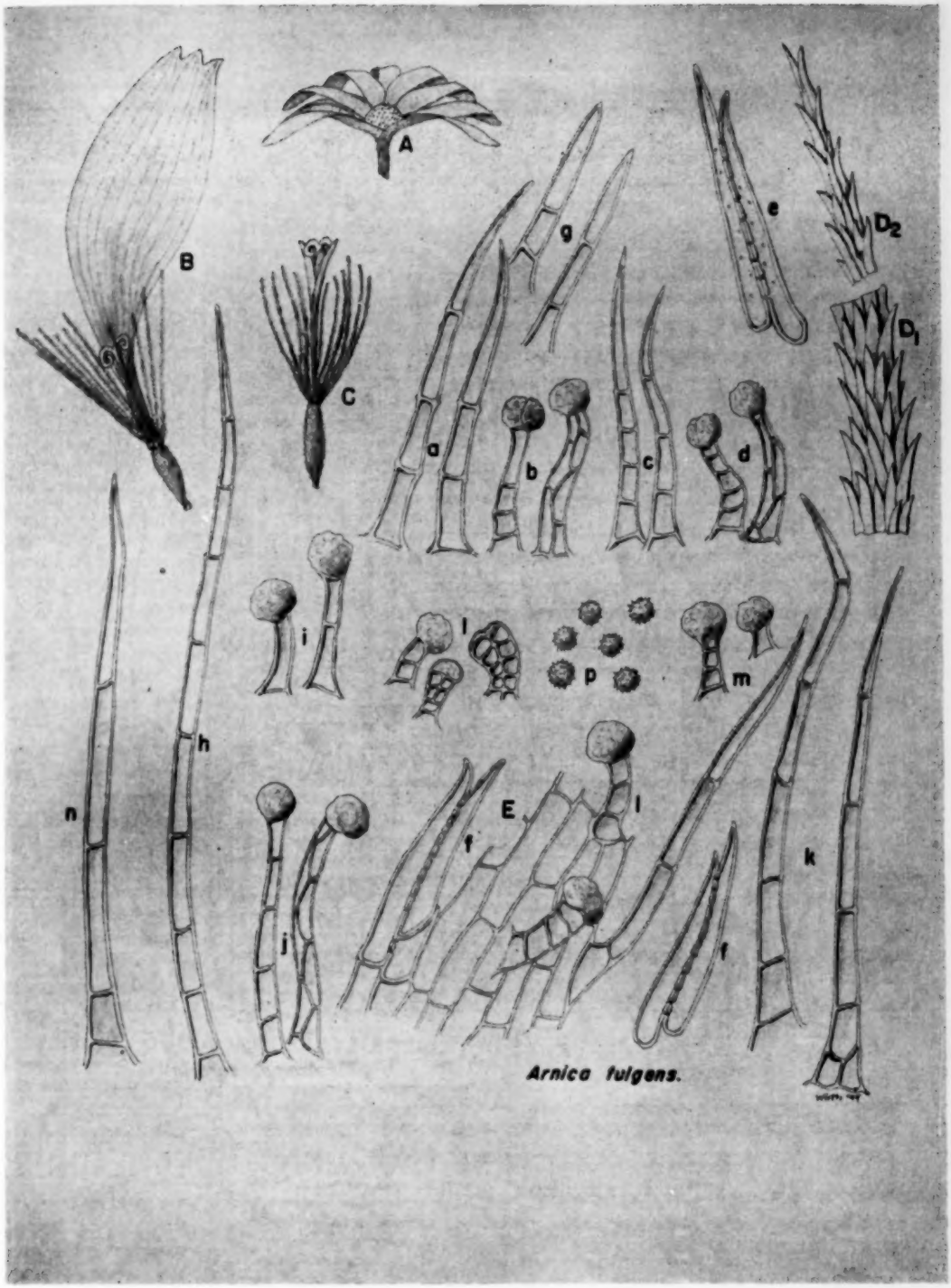
The following microscopical features were observed: the receptacle contains stellate parenchyma and bears on its upper surface numerous unicellular and uniseriate, 2- to 5-celled dagger hairs up to 300 μ in length, the basal cell of the latter being shortest. The margins of the bracts bear two kinds of nonglandular hairs, viz., straight, 1- to 3-celled dagger-shaped hairs and uniseriate, straight or curved, articulate hairs up to 1530 μ in length, the basal cell or cells being shorter than the distal cell or cells. The epidermis of the bracts consists of elongated cells whose long walls are curvilinear. Glandular hairs with a unicellular to uniseriate stalk of up to 4 cells and a 1- to 2-celled head occur along the bract margins. The ligulate corolla bears numerous uniseriate nonglandular hairs with straight to curved end cells, mostly up to 6 cells and 1360 μ in length, also uniseriate dagger hairs, and glandular hairs up to 368 μ in length, with a uniseriate to biseriate stalk and a 1- to 2-celled head. The corolla of the tubular florets possesses lobes with prominent epidermal papillae. The nonglandular hairs of the corolla tube are uniseriate, appressed, up to 6 celled with acuminate-tipped end cell. Short 1- to 2-celled dagger hairs occur in the basal region of the teeth of the tubular corolla. The glandular hairs of the tubular corolla possess a uniseriate to biseriate stalk of up to 8 cells and a 1- to 2-celled head. Along the margin of the receptacle occur slender, articulate, uniseriate nonglandular hairs of up to 10 cells with bent or curved ends. The epidermis of the peduncle exhibits numerous nonglandular, articulate hairs of up to 9 cells and up to 1105 μ in length, also glandular hairs with a uniseriate stalk and a 1- to 2-celled head. The achenes of both kinds of florets bear numerous forward projecting double hairs and glandular hairs with a uniseriate to biseriate stalk and a 1- to 2-celled head. The double hairs are up to 384 μ in length, the glandular hairs up to 91 μ in length.

3. Arnica sororia.—Heads hemispherical, up to 2 cm. in height; involucre of 14 to 18 lanceolate bracts in 2 series, the outer bracts 1.3 cm. in length and up to 2.5 mm. in width, the inner bracts up to 1 cm. in length and up to 2 mm. in width, puberulent and glandular hairy. Receptacle hemispherical, deeply convex, its surface pitted and densely hairy with short hairs between the pits. Ligulate florets 11 to 18, the ligulate corolla up to 2.25 cm. in length, its ligula with 9 nerves and up to 6 mm. in width, with 3 equal or nearly equal teeth up to 1.5 mm. in

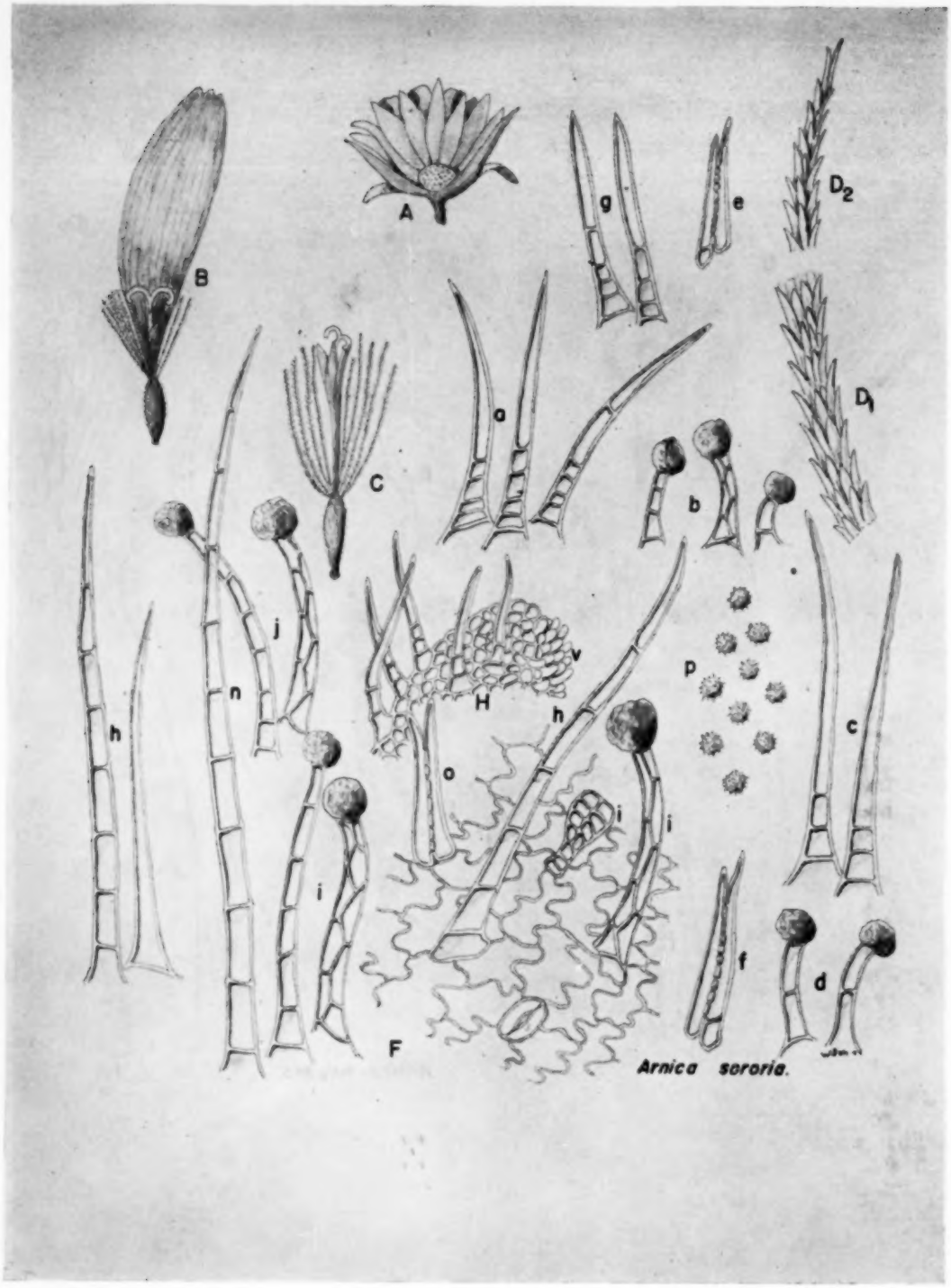


KEY TO DRAWINGS

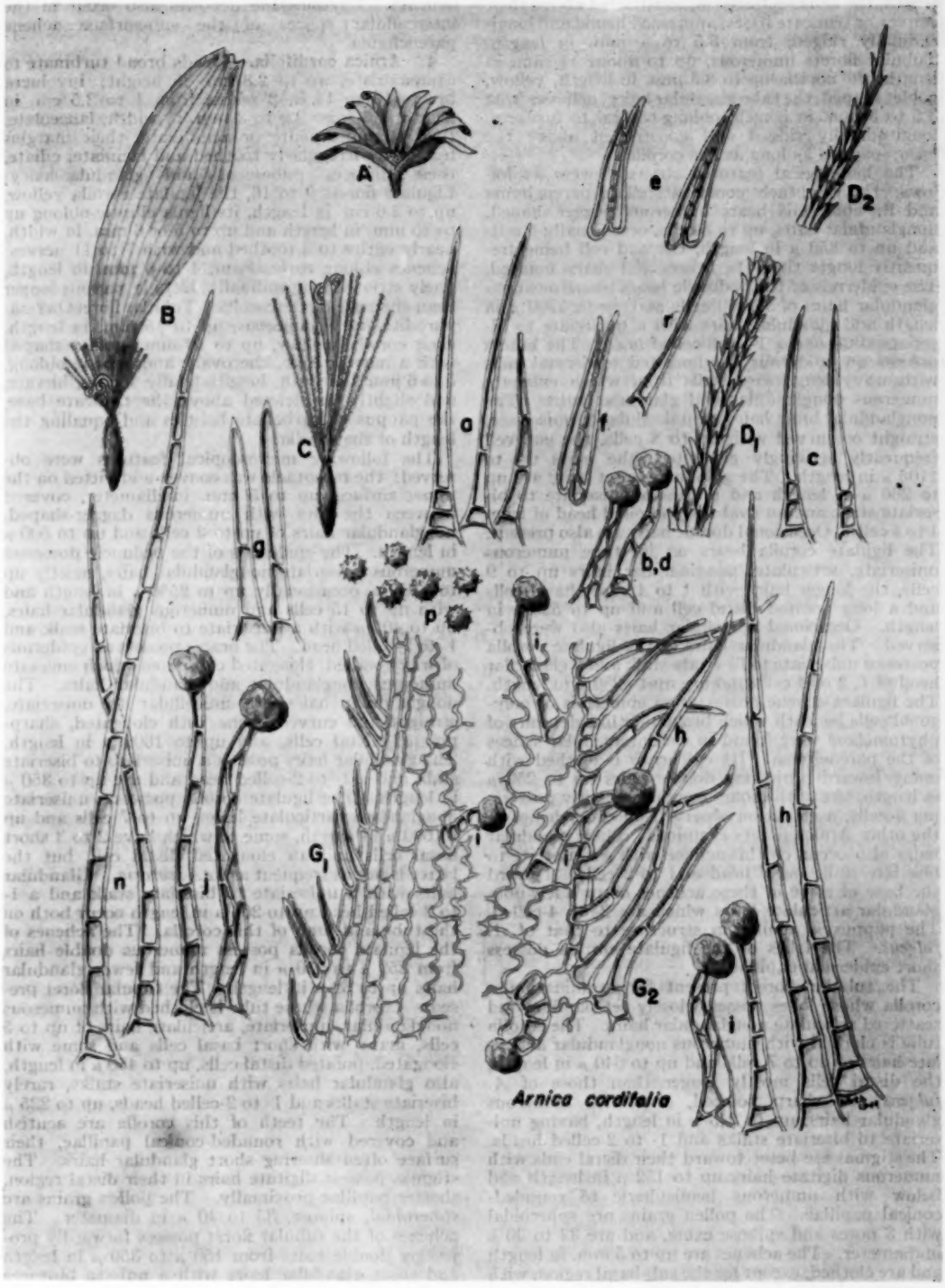
A. Receptacle with bracts.
B. Ligulate floret.
C. Tubular floret.
D_1. Pappus, lower portion.
D_2. Pappus, apical portion.
E. Surface fragment, ligulate achene.



F. Surface fragment, bract.
G_1. Inner bract, margin.
G_2. Outer bract, margin.
H. Tooth, tubular corolla.
a. Nonglandular hair, ligulate corolla.
b. Glandular hair, ligulate corolla.
c. Nonglandular hair, tubular corolla.
d. Glandular hair, tubular corolla.
e. Double hair, ligulate achene.
f. Double hair, tubular achene.



g. Nonglandular hair, receptacle.
h. Nonglandular hair, bract.
i. Glandular hair, bract.
j. Glandular hair, peduncle.
k. Marginal nonglandular hair, receptacle.
l. Glandular hair, ligulate achene.
m. Glandular hair, tubular achene.
n. Nonglandular hair, peduncle.
o. Double hair, bract.
p. Pollen grains.



r. Nonglandular hair, ligulate achene.
s. Stoma.
t. Phytomelane.
u. Nonglandular hair, tubular achene.
v. Papillae.

Receptacle, x 1.15; Ligulate and tubular florets, x 2.3; all other elements, x 77.

length, the achenes fusiform, constricted above their convex or truncate bases, appressed hispid and longitudinally ridged, from 3.5 to 4 mm. in length. Tubular florets numerous, up to about 14 mm. in length, the corolla up to 8.5 mm. in length, yellow, goblet shaped, the tube glandular hairy, achenes from 3.5 to 5 mm. in length, oblong-conical to fusiform, longitudinally ridged and constricted above the base; pappus as long as the corolla.

The histological features observed were as follows: the receptacle contains stellate parenchyma and its epidermis bears numerous dagger-shaped, nonglandular hairs, up to 5 cells, occasionally 6 cells and up to 350 μ in length, the end cell being frequently longer than the others and sharp pointed. The epidermis of the peduncle bears numerous nonglandular hairs of 1 to 9 cells and up to 1700 μ in length and glandular hairs with a uniseriate to biseriate stalk and a 1- to 2-celled head. The bracts possess an epidermis of elongated epidermal cells with wavy lengthwise walls from which emanate numerous nonglandular and glandular hairs. The nonglandular bract hairs are unicellular to uniseriate, straight or curved with up to 8 cells, the end cell frequently strikingly elongated, the hairs up to 1105 μ in length. The glandular bract hairs are up to 266 μ in length and possess a uniseriate to biseriate stalk and an oval to spheroidal head of from 1 to 4 cells. Occasional double hairs are also present. The ligulate corolla bears on its tube numerous uniseriate, articulate, nonglandular hairs up to 9 cells, the longer hairs with 1 to 4 short basal cells and a long pointed distal cell and up to 525 μ in length. Occasional unicellular hairs also were observed. The glandular hairs of the ligulate corolla possess a uniseriate to biseriate stalk and a glandular head of 1, 2 or 4 cells and are up to 250 μ in length. The ligulate achene possesses an epidermis of polygonal cells beneath which broken netlike deposits of phytomelane were found in the intercellular spaces of the parenchyma. Its epidermis is clothed with many foward projecting double hairs up to 250 μ in length, one of the components frequently possessing 2 cells, a condition observed on the achenes of the other Arnica florets examined. Short glandular hairs also occur on this achene with a uniseriate to biseriate stalk and a head of 1 to 2 cells. Toward the base of some of these achenes occur a few nonglandular articulate hairs which are 2- to 4-celled. The pappus is similar in structure to that of *A. fulgens*. The lobes of the ligulate corolla possess short epidermal papillae.

The tubular florets present for examination a corolla whose lobes possess closely set papillae and scattered articulate nonglandular hairs. The corolla tube is clothed with numerous nonglandular articulate hairs of up to 7 cells and up to 640 μ in length, the distal cells mostly longer than those of *A. fulgens* and sharp pointed, also with numerous glandular hairs, up to 243 μ in length, having uniseriate to biseriate stalks and 1- to 2-celled heads. The stigmas are beset toward their distal ends with numerous digitate hairs up to 152 μ in length and below with numerous hemispheric to rounded-conical papillae. The pollen grains are spheroidal with 3 pores and spinose exine, and are 32 to 36 μ in diameter. The achenes are up to 5 mm. in length and are clothed, except for the sub-basal region, with numerous double hairs up to 350 μ in length and with glandular hairs similar to those on the ligulate achenes. Phytomelane deposits also occur in the intercellular spaces of the sub-surface achene parenchyma.

4. **Arnica cordifolia.**—Heads broad turbinate to campanulate, up to 2.8 cm. in height; involucre bracts 10 to 14 in 2 series, from 1 to 2.5 cm. in length and from 0.9 to 3 mm. in width, lanceolate, their summits acute or acuminate, their margins frequently irregularly toothed and laciniate, ciliate, their surfaces pubescent and glandular-hairy. Ligulate florets 9 to 16, the ligulate corolla yellow, up to 2.6 cm. in length, its ligula elliptic-oblong up to 25 mm. in length and up to 5 or 6 mm. in width, nearly entire to 3-toothed and with 7 to 11 nerves; achenes oblong to fusiform, 4 to 6 mm. in length, finely striated longitudinally, hispid; pappus longer than the tube of the corolla. Tubular florets hermaphrodite, very numerous, up to 15 mm. in length, their corollas yellow, up to 10 mm., goblet shaped with a narrow tube, the ovary and achene oblong, 5 to 6 mm. in length, longitudinally striate, hirsute, and slightly constricted above the truncate base, the pappus of barbellate bristles and equaling the length of the corolla.

The following microscopical features were observed: the receptacle was convex and pitted on the upper surface, up to 5 mm. in diameter, covered between the pits with numerous dagger-shaped, nonglandular hairs of up to 3 cells and up to 500 μ in length. The epidermis of the peduncle possesses numerous uniseriate nonglandular hairs, mostly up to 1105 μ, occasionally up to 2550 μ in length and with up to 15 cells and numerous glandular hairs, up to 405 μ with a uniseriate to biseriate stalk and 1- to 2-celled head. The bracts possess an epidermis of wavy-walled, elongated cells from which emanate numerous nonglandular and glandular hairs. The nonglandular hairs are unicellular to uniseriate, straight and curved, some with elongated, sharp-pointed distal cells, and up to 1600 μ in length. The glandular hairs possess a uniseriate to biseriate stalk and a 1- to 2-celled head and are up to 350 μ in length. The ligulate corolla possesses uniseriate nonglandular articulate hairs, up to 7 cells and up to 600 μ in length, some of which have 2 to 3 short basal cells and an elongated distal cell, but the latter is not as frequent as in *A. sororia*. Glandular hairs with a uniseriate to biseriate stalk and a 1- to 2-celled head up to 266 μ in length occur both on the tube and limb of this corolla. The achenes of the ligulate corolla possess numerous double hairs from 250 μ to 350 μ in length and fewer glandular hairs up to 62 μ in length. The tubular floret presents a corolla whose tube is clothed with numerous nonglandular, uniseriate, articulate hairs of up to 5 cells, many with short basal cells and some with elongated, pointed distal cells, up to 450 μ in length, also glandular hairs with uniseriate stalks, rarely biseriate stalks and 1- to 2-celled heads, up to 225 μ in length. The teeth of this corolla are acutish and covered with rounded-conical papillae, their surface often showing short glandular hairs. The stigmas possess digitate hairs in their distal region, shorter papillae proximally. The pollen grains are spheroidal, spinose, 35 to 40 μ in diameter. The achenes of the tubular floret possess forwardly projecting double hairs from 150 μ to 350 μ in length and short glandular hairs with a uni- to biseriate stalk and a 1- to 2-celled head, up to 150 μ in length. Phytomelane occurs in both kinds of achenes.

SUMMARY

1. Descriptions are given of *Arnica montana* L., the principal source of European Arnica imported into this country prior to the present World War, and of three native American species of *Arnica* which represent sources of domestic Arnica in current use in

the United States, namely, *Arnica fulgens* Pursh., *A. sororia* Greene and *A. cordifolia* Hooker.

2. The distribution, habitat and commercial availability of *Arnica montana*, *A. fulgens*, *A. sororia* and *A. cordifola* are cited.

3. The results of comparative studies of the gross structure and of the histology of the flower heads of each of these 4 species of *Arnica* are given by detailed descriptions and 4 plates of drawings.

4. Further studies are contemplated on the oleoresin found in the glandular hairs of the bracts, florets and peduncles of the three American species of *Arnica* discussed in this paper.

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The Inhibitory Effect of Certain Naphthoquinones on the Hemorrhagic Action of Dicumarol*

By D. C. Brodie, W. A. Hiestand, and G. L. Jenkins

Dicumarol, 3,3′-methylenebis(4-hydroxycoumarin), has shown considerable promise as an anticoagulant drug. It was isolated, synthesized and identified in 1941 by Campbell (1) and Link (2) who showed that it was the causative agent of "sweet clover disease." Roderick (3) was the first to show that the hemorrhagic action of spoiled sweet clover hay was due to a reduction of the blood prothrombin level as manifested in a delay of the coagulating time of the blood. Since then this observation has been confirmed both experimentally and clinically.

Early methods of controlling the hemorrhagic diathesis in cattle poisoned with sweet clover hay included transfusions of defibrinated blood (4) and the use of an alfalfa supplement in the diet (5). Since alfalfa was a source of vitamin K, the use of synthetic vitamin K-like substances as a method of counteracting the hypoprothrombinemia was considered a suitable expedient. Overman (6, 7) showed that doses of 2-methyl-1,4-naphthoquinone in greater than therapeutic amounts would partially or completely protect the rabbit and rat from the hemorrhagic action of dicumarol. In addition to feeding 2-methyl-1,4-naphthoquinone, Overman also reported protective action with 2-methyl-1,4-naphthohydroquinone diacetate and 2-methyl-1,4-naphthoquinone-3-sodium sulfonate.

The purpose of this study was to investigate further the inhibiting action of compounds related to vitamin K on the hypoprothrombinemia induced by dicumarol in the rat. The following compounds were selected for study: (*a*) 2-methyl-1,4-naphthoquinone, (*b*) 2-methyl-1,4-naphthohydroquinone, (*c*) the 2,3-oxide of 2-methyl-1,4-naphthoquinone and (*d*) 2-methyl-3-hydroxy-1,4-naphthoquinone (phthiocol). These compounds were synthesized according to the methods of Fieser (8) during the course of a study (unpublished) of certain related naphthoquinones.

EXPERIMENTAL

Methods Used.—Adult female and male albino rats weighing approximately 225 and 325 Gm., respectively, maintained on a diet of Purina Dog Chow Checkers, were used in this study. A 2.5-mg. dose of dicumarol[1] was routinely used in all animals to

* Received July 27, 1944.

This paper is an abstract from Part II of a thesis submitted by D. C. Brodie to the Graduate School of Purdue University in partial fulfillment of the requirements for the Doctor of Philosophy degree.

[1] Supplied through the courtesy of Abbott Laboratories, Chicago, Ill.

produce the prothrombinopenic state. The inhibiting quinones and the dicumarol were incorporated in 2 cc. of freshly prepared mucilage of acacia (U. S. P. strength, without the preservative) and administered simultaneously by means of a stomach tube, attached to a suitable hypodermic syringe. Animals were fasted for twelve hours before receiving the drug portions; when a group of animals was being used in consecutive experiments, a rest period of seven to ten days was permitted between experiments. The degree of prothrombin depletion was obtained in terms of the prothrombin time of 12.5 per cent plasma according to the Link modification (9) of the Quick method. Blood samples were obtained by heart puncture, under light ether anesthesia, twenty-four hours after the drug feeding since dicumarol exhibits its maximum hypoprothrombinemic effect in the rat at this time. Three clotting times for each plasma sample were obtained and the arithmetical mean of these was chosen as the prothrombin time. Dehydrated rabbit brain tissue was prepared according to Link and stored in an atmosphere of nitrogen in 0.1- and 0.15-Gm. portions in sealed one-dram vials at 0° C. The brain tissue stored under these conditions maintained remarkably constant thromboplastic activity during a nine-week period in which the activity was assayed weekly.

Results.—Normal prothrombin times were determined for 7 groups of rats totaling 78 animals. The group averages ranged from 39.1 seconds to 47.8 seconds, the average animal prothrombin time being 44.9 seconds. Normal values previously observed (7, 10) corresponded with those herein reported. Normal prothrombin times in a limited number of animals were also obtained in which Russell viper venom[2] was substituted for the tissue extractive as a source of thromboplastin. The venom was prepared according to the directions of the manufacturer for a 1:10,000 solution and was used in the same amounts as the extractive prepared from rabbit brain. Normal prothrombin times averaged 19.3 seconds with the venom solution whereas the prothrombin times of the same plasma samples determined with tissue extractive averaged 49.6 seconds.

The 2.5-mg. dose of dicumarol increased the prothrombin time to 126 seconds, twenty-four hours after its administration. This dose of dicumarol produced similar results in both male and female rats although the weight variation was about 100 Gm. The amounts of protection in terms of prothrombin time, which the vitamin K-like substances provided against the hypoprothrombinemia, are recorded in Table I.

TABLE I.—MODIFICATION OF DICUMAROL ACTION BY NAPHTHOQUINONES

Drug	Dose of Drug	No. of Rats	Prothrombin Time, Sec.
Dicumarol	2.5 mg.	31	126.9
Phthiocol	75 mg./Kg.	23	98.1
2,3-Oxide of 2-methyl-1,4-naphthoquinone	75 mg./Kg.	13	89.9
2-Methyl-1,4-naphthohydroquinone	75 mg./Kg.	13	77.5
2-Methyl-1,4-naphthoquinone	75 mg./Kg.	29	74.0
2-Methyl-1,4-naphthoquinone	37 mg./Kg.	18	78.1
2-Methyl-1,4-naphthoquinone	150 mg./Kg.	18	82.6
Controls	.....	78	44.9

DISCUSSION

Since 2-methyl-1,4-naphthoquinone is frequently used as a standard in the assay of vitamin K-like substances, this compound was used as a basis for measuring the relative inhibiting action of the several compounds used in this study. Overman (7) reported complete protection against 2.5-mg. doses of dicumarol in the rat with 25-mg. doses of 2-methyl-1,4-naphthoquinone. Early observations in the present study indicated that when the dose of this quinone was considered on a body-weight basis the antihemorrhagic action might be inversely related to the dose. It was found that this relationship did exist when male and female animals of a uniform weight were fed large and small doses of the antihemorrhagic compound. Maximum protection amounting to a 65 per cent reduction of the prothrombin time from the hypoprothrombinemic state was obtained when the naphthoquinone was administered on a 75 mg./Kg. of body-weight basis. Meunier, *et al.* (11), in 1940 observed the same inverse relationship during a photometric study of the antihemorrhagic activity of 2-methyl-1,4-naphthoquinone in avitaminotic K-rabbits. The work which is herein reported confirms the report of the French workers, although at the time that this conclusion was drawn, the report of Meunier had not been observed in the literature. The significance of this observation lies in the fact that vitamin K therapy is one of the possible treatments for clinical patients who receive excessive amounts of dicumarol. A potentiating rather than an inhibiting effect could be obtained if the attending physician did not realize that large amounts of 2-methyl-1,4-naphthoquinone might aggravate an existing hemorrhagic condition.

One of the objectives of this study was to determine whether the vitamin K-like substances used in this work exerted the same relative antihemorrhagic activity against dicumarol as they do in counteracting the antihemorrhagic condition in avitaminotic K-chicks. Fieser (12) assayed a large number of the synthetic antihemorrhagic compounds and reported the following order for those substances which are considered in this

[2] "Stypven," supplied by Dr. C. J. Klemme, Burroughs Wellcome & Co., Tuckahoe, N. Y.

paper. The value in parentheses indicates the relative amount needed to produce antihemorrhagic activity of the compound as compared to synthetic vitamin K_1, 2-methyl-1,4-naphthoquinone (0.3), 2-methyl-1,4-naphthohydroquinone (0.5), vitamin K_1 (1), the 2,3-oxide of 2-methyl-1,4-naphthoquinone (5), and phthiocol (500). The data presented in Table I show that the same order of activity exists when these compounds are fed antagonistically to dicumarol as when they are fed to avitaminotic K-chicks. In the chick studies phthiocol exerted such a slight antihemorrhagic activity that the effect of the same compound in counteracting the hypoprothrombinemia resulting from dicumarol was expected to be practically negligible. However, phthiocol inhibited the activity of dicumarol to a surprisingly high degree, the inhibition amounting to approximately 35 per cent (based on the reduction of prothrombin times) as compared to 65 per cent inhibition which the same dose of 2-methyl-1,4-naphthoquinone afforded.

CONCLUSIONS

1. The inhibiting effect of 2-methyl-1,4-naphthoquinone, 2 - methyl - 1,4 - naphthohydroquinone, 2,3-oxide of 2-methyl-1,4-naphthoquinone and phthiocol on the action of dicumarol in the rat is reported. Of these, 2-methyl-1,4-naphthoquinone is most active, with a dose of 75 mg./Kg. of body weight being more effective in counteracting the hypoprothrombinemia than either a 37 mg. or a 150 mg./Kg. of body weight dose.

2. Phthiocol, when assayed against dicumarol in the rat, shows considerably greater antihemorrhagic activity than it does in avitaminotic K-chicks. When compared with 2-methyl-1,4-naphthoquinone, phthiocol was approximately one-half as effective in counteracting the action of dicumarol.

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WANTED—1944 PROCEEDINGS ISSUE

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Solubility, Density and Melting Point of Phenolphthalein*

By Max H. Hubacher†

It is surprising that no accurate figures on some of the properties of phenolphthalein are available. This lack of definite information is more noteworthy when we consider the importance and wide use of this drug. The solubility of phenolphthalein in water at room temperature is given by Dehn (1) as 1:2500; by Zotier (2) as 1:10,800; by McCoy (3) as 1:37,735; by Utz (4) 1:50,000; and by Fantus and Dyniewicz (5) as 1:333,333. Utz (4) mentions the solubility of phenolphthalein in a dozen solvents but does not specify their purity nor the temperature at which these determinations were made. Fantus and Dyniewicz (5) record the solubility of phenolphthalein at 25° C in water, 18 organic solvents and 8 different oils. They state that the solubility in aqueous alcohol increases with the concentration up to the official alcohol, but is slightly less in absolute alcohol, and that the solubility in U. S. P. ether is more nearly 1 Gm. in 100 cc. This was confirmed in our laboratory. However, their data would have been more valuable if they had specified the purity of the solvents.

According to U. S. P. XII, 1 Gm. phenolphthalein is soluble in 15 cc. alcohol and in 75 cc. ether at 25° C, while in U. S. P. X and U. S. P. XI it was "in 13 cc. alcohol and in about 70 cc. ether at 25° C." Based on the data obtained, shown in Table I, and confirmed by Fantus and Dyniewicz (5), it is proposed that these figures be changed and the solubility in four other solvents noted. It is suggested that the paragraph on "Solubility" in U. S. P. XII be changed to read "Phenolphthalein is practically insoluble in petroleum ether, carbon tetrachloride, benzene and water. One gram of it dissolves in 5 cc. acetone, 13 cc. alcohol and 110 cc. ether at 25° C."

The density of phenolphthalein is given in textbooks (6) as 1.277. Repeated determinations made in this laboratory show a value of $d\frac{25°}{4°} = 1.299$.

The melting point of phenolphthalein is still often quoted at 250–253° which is the melting point recorded by its discoverer, A. Baeyer (7). U. S. P. XII states that phenolphthalein should not melt below 258°. Bebie (8) found the melting point of domestic and foreign samples to lie between 261.2° and 262.8°. Phenolphthalein sublimed *in vacuo* is said to melt at 260.6–261.5° (9). Analysis of 104 lots of domestic U. S. P. phenolphthalein sold by the two principal manufacturers of this drug since 1937 showed an average melting point of 261.2–261.9°, with none starting to melt below 260.4°. It is suggested that the specifications in U. S. P. XII be changed to read "melts between 259° and 263°."

EXPERIMENTAL[1]

Solubility.—The solvent, containing an excess of phenolphthalein U. S. P. XII, m. p. 261.0–261.9°, loss on drying 0.015%, was placed in a cork- or glass-stoppered flask and set into a water bath thermostatically controlled at 25.0° ± 0.3°. In another series of experiments, a warm, saturated solution of the same phenolphthalein in the solvent was sealed under CO_2 into a large Pyrex test tube and this likewise was placed in the water bath.

The flask and tubes were shaken occasionally. Before aliquots were removed, the flasks were left in the bath for at least one week, and the tubes one month. Additional samples were pipetted at intervals of three days to a week. Analysis of the solutions kept for longer periods would indicate whether or not solubility equilibrium was attained. In most cases, this point was reached after one week. The data in Table I are the average of at least ten determinations, except in the case of petroleum ether, carbon tetrachloride, glycerin and benzene, where only five determinations were made.

Analytical procedure consisted of pipetting from 3 to 20 cc. of the supernatant, clear solution into a weighing bottle, the liquid weighed, evaporated and the residual phenolphthalein dried to constant weight at 130°. In the case of ethylene glycol and pyridine, the solution was evaporated at 150° with the aid of a stream of dry air and the residual phenolphthalein dried at 220°. When the supernatant liquid was not clear, the suspension was filtered by gravity in a small filter apparatus with a sintered glass plate, enclosed to prevent evaporation and thus avoid supersaturated solutions.

Because of the slight solubility of phenolphthalein in water, these determinations were made differently, as follows: Solutions of 2 Gm. phenolphthalein in 10 liters of boiling, distilled water were prepared, and about 200 liters of this solution was stored close to 25° for at least one month, during which time phenolphthalein crystallized out. From 10 to 35 liters of the doubly filtered solution were evaporated under partial pressure on a water bath in a 1-liter, round-bottom flask fitted with a dropping funnel through which the solution was added gradually. The phenolphthalein remaining in the flask was dissolved in about 10 cc. 5 *N* sodium hydroxide and

* Received Aug. 18, 1944, from the Research Laboratory of Ex-Lax, Inc., Brooklyn 17, N. Y.

† The author is indebted to A. Horner for assistance in these determinations.

[1] All melting points are corrected.

TABLE I.—SOLUBILITY OF PHENOLPHTHALEIN AT 25° C.

Solvent					
Name	Grade	Spec. Gr. $\frac{25°}{4°}$	1 Gm. Dissolves in Solvent[c] Gm.	Cc.	100-Gm. Solvent Dissolves Gm.
Pyridine[a]	Anhydrous	0.9789	0.657 ± 0.021	0.670	152.29
Acetone, U. S. P. XII	Boiling point, 55.5–55.8°	0.7856	3.57 ± 0.15	4.54	28.01
Ethanol, U. S. P. XII	92.4%	0.8070	9.64 ± 0.26	11.94	10.37
Ethanol, absolute, U. S. P. XII	99.6%	0.7861	10.86 ± 0.02	13.82	9.20
Methanol	Anhydrous	0.7868	10.90 ± 0.07	13.85	9.17
Ethylene Glycol	Boiling point, 195–197°	1.1111	46.60 ± 1.18	41.94	2.14
Ether, U. S. P. XII	"Mallinckrodt," Control, 702393	0.7132	71.23 ± 3.14	99.87	1.40
Ether, U. S. P. XII	96.8% ether, 2.9% C_2H_5OH and 0.2% water	0.7121	73.65 ± 0.43	103.42	1.36
Ethanol, diluted, U. S. P. XII	41.5%	0.9283	127.07 ± 1.09	136.88	0.78
Acetic Acid, U. S. P. XII	99.7%; congealing temp., 16.0°	1.0441	223.9 ± 5.4	214.4	0.44
Ether	Pure; distilled several times over metallic sodium	0.7078	205.8 ± 3.7	290.7	0.48
Glycerin, U. S. P. XII	95.2%	1.2454	1,911 ± 279	1,534.0	0.053
Chloroform	Free of alcohol; purified according to *J. Am. Chem. Soc.*, **60**, 2560(1938)	1.4754	15,951 ± 1,792	10,811	0.006
Benzene	Congealing temp., 5.2°	0.8714	31,410 ± 2,261	36,045	0.0031
Water[b]	Distilled	...	383,600 ±23,000	...	0.0002
Carbon Tetrachloride, U. S. P. XII	Purified as was the chloroform	1.5836	More than 5 million	More than 3 million	...
Petroleum Ether	Boiling range, 35–60°	0.6342	More than 5 million	More than 7 million	...

[a] The pyridine solution is colorless.
[b] One gram phenolphthalein dissolves in about 4200 cc. water at 100°.
[c] Average and mean deviation.

TABLE II.—MELTING POINT AND DENSITY OF PHENOLPHTHALEIN

Product	Melting Point °C.[a]	Melting Point °C.[b]	Medium	Density $\frac{20°}{4°}$
U. S. P. XII, Monsanto Chem. Works, Lot B-106-A	260.9–261.6	260.8–261.7	Toluene	1.2976
U. S. P. XII, Mallinckrodt Chem. Works, control WNBS	260.7–261.4	260.8–261.6	Toluene	1.2986
U. S. P. XII, Chas. Pfizer, Inc., Lot 42,970	261.1–261.9	260.8–262.0	Water	1.2958
Precipitated[c]	260.2–261.3	260.5–261.7	Water	1.3001
Crystals from acetic acid	260.0–262.1	260.1–261.6	Water	1.3008
Crystals from alcohol	260.8–261.5	260.4–261.1	Water	1.3009
			Av. =	1.2989[d]

[a] Determined in a Hershberg precision melting point apparatus according to the method described in the text.
[b] Determined by the U. S. P. XII method, using the official thermometer II.
[c] Prepared by precipitating 100 Gm. phenolphthalein, U. S. P. XII, according to the example on page 4, lines 68–88 in U. S. Patent 1,940,495.
[d] F. D. Smith of Monsanto Chemical Works, St. Louis, Mo., obtained an average density value of 1.298 on several samples of U. S. P. phenolphthalein (private communication of March, 1944).

determined gravimetrically by the tetraiodo method (10). A similar procedure was used in the determination of the solubility in chloroform and benzene, using 3 Kg. solution for each determination. The solubility in glycerin was determined by distilling off the solvent in high vacuo from an oil bath heated to 210°. The phenolphthalein was determined in the residue by the tetraiodo method.

The solubility in petroleum ether and carbon tetrachloride was determined by evaporating about 3 Kg. of the saturated solution, taking up the residue in a buffer solution of *p*H 10.0 and determining the phenolphthalein therein colorimetrically within a few minutes, using 50 cc. Nessler tubes (12).

Density.—The density of phenolphthalein was determined on about 6-Gm. samples in a 50-cc. pycnometer, using either toluene or distilled water containing 0.03% of detergent "7027D."[2]

Melting Point.—The melting point was determined in a Hershberg precision melting point apparatus (11) with a thermometer range of 200° to 270°,

[2] Sold by Palo-Myers, Inc., New York, N. Y.

subdivided into 0.2° and having 5-cm. immersion. This thermometer was checked frequently with a similar one corrected by the National Bureau of Standards. The capillary tube of U. S. P. XII dimensions was introduced into the bath when the temperature reached 258° and the rise in temperature was regulated to one-half degree per minute. The same values were obtained with this method as with the procedure described in U. S. P. XII, page 596, which calls for introducing the capillary tube 30° below the melting point and heating at a rate of 3° each minute until the substance starts to melt, then raising the temperature one-half degree per minute. It is difficult to follow this U. S. P. method exactly due to the sudden change in the rate of heating. The method detailed above is quicker and more accurate, especially for substances having a high melting point. The melting points of the 104 lots of commercial U. S. P. phenolphthalein mentioned in the introduction were determined in this manner.

SUMMARY

The density of phenolphthalein, as well as the solubility in many common solvents, was determined.

Several changes and additions were recommended for U. S. P. XII description of the solubility and melting point of phenolphthalein.

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A Phytochemical Investigation of *Larrea Divaricata* Cav.*

By Coy W. Waller† and Ole Gisvold‡

Larrea divaricata Cav. Fam. *Zygophyllaceae* is a shrub that is indigenous to some of the southwestern states, i.e., Texas, Nevada, New Mexico, Utah, Arizona and Mexico. It is well recognized by the natives under the common name, creosote bush. It derives this common name because of an abundance of a resinous coating on the leaves and small twigs, particularly at the nodes, and because the shrub when burned yields a black smoke having a rank odor resembling creosote. This shrub is known as "el gobernadora" and "hideonodo" by the Spanish Californians. It is also known by the natives as palo ondo, tasajo, gumis, hediondilla, sonora, falsa, al caparra, huamis, yah temp and etiontio. This shrub is also known as *Covillea tridentata*, (D. C.) Vail, and adequate botanical descriptions can be found in the literature (1).

Larrea divaricata Cav. belongs to a relatively small family, i.e., *zygophyllaceae*. *Guajacum officinale* Linne and *Guajacum sanctum* Linne also belong to this family. The N. F. Guaiacum which is a resin is obtained from these species of *Guajacum*.

Chemical investigations of *Larrea divaricata* have been limited primarily to selective solvent extractions of the crude drug (2), solubility constants of the resin exudate (3), ash determinations (4) together with the percentage of some of the elements in the ash (5).

Larrea divaricata has been used by the Indians (6) of the southwest for many ail-

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ments. A tea from boiled leaves is drunk for venereal diseases, colds, and bowel cramps and to stimulate urination. The leaves are soaked in water to produce an extract which is used as a bath for rheumatism and chickenpox. The dried powdered leaves are used as a dusting powder for sores. A condensed decoction is mixed with badger oil to make an ointment which is used in burns to aid in the formation of new skin.

The following medicinal claims and uses have been made for this plant: healing sores and wounds, rheumatism, inflammation of the respiratory and intestinal tract, tuberculosis, gastric disturbances, venereal diseases, tonic, corrective, antiseptic, stimulating expectorant, and emetic.

No detailed pharmacological investigation of this plant or its resinous exudate has been reported in the literature.

Larrea divaricata has recently become of an ecological interest because it can be used in the prevention of soil erosion. It grows best on well-drained upland soil in the southwestern part of the United States in a region where soil erosion is a great problem. It grows on arid soil and does not need irrigation.

The plant material used in this investigation was obtained through the Indian Drug Plant Project set up in Reno, Nev., by the Division of Plant Exploration and Introduction, Bureau of Plant Industry, United States Department of Agriculture.

EXPERIMENTAL

The plant material used in this investigation consisted of small twigs, leaves, flowering tops that were, when air dried, ground to a No. 20 powder in a Jacobson laboratory mill. The powdered material was a yellowish green color and had a characteristic aromatic odor which was quite pleasant when not too concentrated.

An alcoholic extract made by exhaustive percolation with 95 per cent alcohol was very active bactericidally against gram-positive bacteria. The alcoholic extract was divided into suitable portions which were treated by extraction with warm water, ether, petroleum ether and chloroform. Bactericidal activity was residual in the ether- and chloroform-soluble fractions. The ether-insoluble fraction was inactive whereas the chloroform-insoluble fraction still retained bactericidal properties.

The marked bactericidal activity of the alcoholic extract was shown to be due to nordihydroguaiaretic acid which was isolated in the following ways.

Lead Method.—The chloroform-insoluble alcoholic extract, which had marked bactericidal activity, was dissolved in alcohol and treated with an alcoholic solution of lead acetate until no further precipitation occurred. The precipitate was collected on a filter and washed free of lead acetate with alcohol. The lead precipitate was suspended in alcohol and hydrogen sulfide gas passed into the suspension until all lead present was converted to the sulfide. The lead sulfide was removed by filtration and washed with alcohol. The lead-free filtrate and washings were concentrated to a small volume, diluted with water, and allowed to stand overnight. A small amount of reddish needles had separated that melted at 176° C. and gave an intense green color with ferric chloride test solution. These crystals were soluble in alcohol and ether, but were insoluble in water and petroleum benzin. The yield of crystalline material by this procedure was not very promising as a large amount of tarry material was obtained.

Ether Petroleum Benzin Procedure.—The ether-soluble fraction of the chloroform-insoluble alcoholic extract was treated with Norite and filtered. The filtrate was poured into a large volume of boiling petroleum benzin from which a fine yellow powder separated that melted at 165° C. and gave a characteristic green color with ferric chloride T. S. The yellow color was removed by treatment with hot chloroform and the resulting product was a cream-colored powder which melted at 180° C. This material, when tested for its bactericidal activity, showed it to be very effective.

Hot Water Procedure.—One thousand and forty-five (1045) grams of an alcoholic extract which represented 30.13 per cent of the powdered plant material was placed in a 5-liter flask and subjected to steam distillation. The distillate was extracted with ether to yield 3 cc. of an oil that was not subjected to further investigation. The flask, after standing overnight, contained two layers, a semi-solid dark extract and a supernatant aqueous layer. The aqueous layer contained crystalline material, which was removed by decantation. The impure crystalline material gave a green color with ferric chloride T. S.

An attempt to prepare more of the crystalline material by treating the solid extract with boiling distilled water was not successful. However, if several grams of sodium bisulfite per liter were used, crystalline material was obtained upon cooling these aqueous extracts.

The crystalline phenolic material obtained in the above procedure contained a relatively large amount of impurities, chiefly consisting of a yellowish green oily solid that passed through the filter paper, while hot, as an oil. This oily material was removed when the filter paper had been previously coated with charcoal or some other filter aid. The resulting product varied in color from a dirty yellow to a reddish brown. Repeated crystallization from hot bisulfite solution finally yielded a crystalline creamy white material which melted at 184° C. This procedure was tedious and accompanied by a large loss of material.

Attempted crystallizations of the crude crystalline material from hot solvents caused the formation of tarry products. The crude crystalline material gave a desirable crystalline material when it was dissolved in hot 36 per cent acetic acid and treated with charcoal, m. p. 184–185° C. Extraction with 10 to 15 per cent acetic acid of the crude alcoholic extract also gave quite satisfactory results for the preparation of the crystalline material.

Identification of the Crystalline Phenol as Nordihydroguaiaretic Acid

Solubility.—The phenol was soluble in ethanol, methanol and ether; slightly soluble in hot water and chloroform; insoluble in petroleum benzin, benzene, toluene and cold water. It is soluble in dilute alkalies, the alkaline solution rapidly changing to a deep red color. It is insoluble in dilute hydrochloric acid and soluble in concentrated sulfuric acid.

Color Reactions, etc.—A few milligrams of the phenol when dissolved in alcohol gave a deep bril-

liant green color with ferric chloride T. S. In acid solution the green color soon changed to yellow. The dropwise addition of a dilute solution of sodium carbonate restored the green color, and further addition of the carbonate caused the solution to change from green to blue, to violet, to purple, and finally to a wine-red color.

A 1 per cent solution of the phenol in acetone decolorized an acetone solution of potassium permanganate to yield a deep brilliant green colored solution.

A 1 per cent solution of the phenol in chloroform decolorized added bromine in chloroform with the evolution of hydrogen bromide.

A few milligrams of the phenol in 2 cc. of absolute ethanol gave immediately an intense blue color on the addition of 2 cc. of sodium ethoxide (2 Gm. of metallic sodium in 50 cc. of absolute ethanol). On the addition of water, this color changed to red. Ether precipitated the blue compound.

To a 2 per cent solution of the phenol in ethanol was added a trace of blood followed by several drops of 3 per cent hydrogen peroxide. A red color developed in ten to fifteen minutes.

Derivatives

Acetate.—A crystalline acetate was readily prepared by the usual methods of acetylation. The crude acetate was dissolved in ethanol, treated with charcoal and recrystallized several times from ethanol, m. p. 102–103° C. The acetate was soluble in ethanol, ether and chloroform; insoluble in water; and slightly soluble in benzene and petroleum benzin.

The acetate was hydrolyzed by a hot 2 per cent solution of hydrogen chloride containing a sufficient amount of ethanol to maintain a clear solution. The regenerated crystalline phenol melted at 185° C.

Anal.—Calcd. for $C_{28}H_{30}O_8$: C, 66.35; H, 6.43. Found: C, 66.55, 66.78; H, 6.87, 6.56.

Methylated Phenol.—The phenol was methylated in the absence of oxygen from the air by means of dimethyl sulfate and alkali. The methylated product was recrystallized from dilute methanol to give crystalline needles that melted at 102–103° C. Methylation with diazomethane gave a product that melted at 99° C. and was not quite so pure as the above product. The methylated phenol was a white, needle-shaped, crystalline compound; insoluble in water; slightly soluble in cold methanol, ethanol, benzene and petroleum benzin; soluble in ether, chloroform, and acetone.

Anal.—Calcd. for $C_{22}H_{20}O_8$: C, 73.70; H, 8.44; mol. wt. (Rast), 358.24. Found: C, 73.82, 73.93; H, 8.23, 8.37; mol. wt. 376. Calcd. for methoxy: 34.61. Found: 34.75, 34.50, 34.82.

Ethylated Phenol.—The phenol was ethylated by means of diethyl sulfate and alkali in the absence of oxygen of the air to yield an ethylated derivative which, after several crystallizations from dilute ethanol, melted at 89–91° C.

Vacuum Sublimation of the Phenol.—Several grams of the phenol were subjected to vacuum sublimation for about six hours at 290–320° C., and a pressure of about 3 mm. mercury. Three products were obtained. The upper zone gave a crystalline material that melted at 104° C., and showed no depression when mixed with pure catechol and melted. The second product was too small to be identified, whereas the third product was the unchanged original material.

The phenolic compound and its derivatives did not contain any solvent of crystallization, because samples showed no loss in weight when dried at 100° C. over phosphorus pentoxide in the Abderhalden drying pistol.

Elementary analysis indicated the absence of sulfur, nitrogen and halogen.

Anal.—Calcd. for $C_{18}H_{22}O_4$: C, 71.48; H, 7.34. Found: C, 71.68, 71.48; H, 7.18, 7.39.

The analytical data obtained for the phenol, the acetylated phenol and the methylated phenol agree with that in the literature (7) for nordihydroguaiaretic acid. A sample of nordihydroguaiaretic acid prepared from resin guaiac was mixed with the phenol and no depression in melting point was obtained.

Petroleum Benzin Extract.—About 3 Kg. of the powdered drug was extracted exhaustively with petroleum benzin to yield about 5.94 per cent extractive matter.

Wax Constituents.—During the extraction with petroleum benzin, a waxlike material separated. This waxlike material was collected on a filter and washed with cold petroleum benzin. The granular-like waxy material was recrystallized from hot ethyl acetate until a constant melting point of 79–80° C. was obtained.

The waxlike material contains no wax alcohols as shown by the fact that acetylation did not alter its melting point.

Saponification (8) of the wax with a mixture of benzene and alcoholic potassium hydroxide yielded an acid and an alcohol. Both acid and alcohol were readily recrystallized from isopropyl ether. The acid melted at 71–74° C. and the alcohol at 85–87° C. Esterification of the acid with diazomethane yielded a methyl ester that was crystallized from methanol and melted at 62–63° C. The alcohol was acetylated with boiling acetic anhydride and pyridine. The resulting acetate was readily crystallized from isopropyl ether and melted at 65–67° C.

The petroleum benzin extract after removal of the solvent was dissolved in a large volume of hot ethanol. A small residue which was not soluble in the hot ethanol had rubberlike properties. Upon cooling, the alcoholic solution deposited impure waxy material which proved to be identical to the above characterized material.

Yellow Pigment.—The above alcoholic solution was concentrated to a volume of about 200 cc. and then allowed to evaporate spontaneously at 25° C. A yellow crystalline material was deposited in this mass. The syrupy mass was mixed with benzene and filtered, whereupon the yellow crystalline material could be collected and washed with cold benzene. These yellow crystals were crystallized from hot ethanol after decolorization with charcoal; m. p. 217–218° C.

The following qualitative tests were applied:

Ferric chloride in alcohol gave a deep yellow color.

Soluble in alkali and gave a yellow solution which apparently did not bring about any degradation.

A few milligrams in glacial acetic acid gave an intense lemon color upon the addition of a few drops of concentrated sulfuric acid. This resulting lemon-colored reaction mixture exhibited a blue fluorescence in sunlight.

A positive reduction reaction (9) with magnesium and hydrochloric acid as characterized by a wine-red color was obtained. This indicated that a flavonol or flavanone type structure was present.

A positive Wilson's boric acid test (10) was obtained. This indicated a hydroxyl group in position 5 of the flavonol type compound.

A few crystals when dissolved in concentrated sulfuric or hydrochloric acid gave an intense lemon-yellow color.

Isolation of a Phytosterol.—The petroleum benzin extract from which most of the wax and yellow pig-

ment had been removed was saponified, and the nonsaponifiable fraction obtained in the usual manner. The nonsaponifiable fraction was quite dark. It was dissolved in hot ethanol from which upon cooling wax alcohol separated. Concentration of the mother liquor yielded more crystalline material which was decolorized by charcoal in alcohol. After several recrystallizations from diluted alcohol, shiny scalelike crystals were obtained that melted at 126–128° C. These crystals gave a positive Lieberman–Burchard color test for sterols. They yielded an acetate which melted at 117–119° C.

Ether Extract.—The dried marc from the petroleum benzin extraction was extracted in a Soxhlet type apparatus with ether.

Orange Pigment.—An orange-colored, crystalline substance separated from the above ether extract after standing several weeks to permit spontaneous evaporation of part of the solvent. The orange crystals were collected on a filter and washed with cold ether. They were contaminated with waxy material which was removed by treatment with hot 50 per cent ethanol in which the wax was insoluble. Several recrystallizations from diluted ethanol gave orange, needle-shaped crystals which melted at 230–233° C. with decomposition.

The following qualitative tests were applied:

Ferric chloride in alcohol gave a dirty red color.

Soluble in alkali to give a deep orange color which apparently did not bring about any degradation.

A few milligrams in glacial acetic acid gave an intense reddish orange color upon the addition of a few drops of concentrated sulfuric acid. This solution possessed no fluorescence in sunlight.

The reduction reaction with magnesium and hydrochloric acid was positive (*loc. cit.*).

A positive Wilson's boric acid test was obtained (*loc. cit.*).

A negative Molish test was obtained.

Elementary analysis of this orange pigment indicated the absence of sulfur, nitrogen or halogen.

Anal.—Calcd. for $C_{18}H_{16}O_8$: C, 60.00; H, 4.44. Found: C, 60.21, 60.26; H, 4.68, 4.77.

Isolation of Sucrose.—The water-soluble fraction from the alcoholic extract was dissolved in methanol. Upon spontaneous evaporation, crystalline material was deposited in a syrupy residue. The crystalline material was separated and crystallized several times from absolute methanol, m. p. 185° C., $[\alpha]_D^{25} = 65°$. The substance was sweet to the taste and did not reduce Fehling's Solution until after hydrolysis. The substance must, therefore, be sucrose.

Tests for Alkaloids.—The usual procedures used for testing for the presence of alkaloids were employed. All results were negative.

Volatile Oil Content.—A determination of the volatile oil showed the presence of 0.1 per cent.

SUMMARY AND CONCLUSIONS

One, if not the most active, of the bactericidal agents present in *Larrea divaricata* Cav. has been characterized as nordihydroguaiaretic acid, m. p. 184–185° C. This is the first recorded instance of the isolation of this compound in nature.

A wax has been isolated that is composed of esters. Saponification yielded an acid and an alcohol fraction. The melting point of the acid fraction indicates it to be a mixture of C_{28} and C_{26} acids. The alcohol fraction appears to consist mainly of a C_{30} alcohol.

The nonsaponifiable portion of the petroleum benzin extract yielded a small amount of a sterol fraction which melted at 126–128° C. and was apparently a mixture.

A yellow flavonol with an auxochromic group in position 5 has been isolated and partially characterized.

An orange-colored flavonol that has an auxochromic group in position 5 has been isolated and partially characterized.

Although *Larrea divaricata* has a marked characteristic aromatic odor, only a very small amount of volatile oil has been obtained upon steam distillation.

Although alkaloids have been purported to be present in *Larrea divaricata*, the usual alkaloidal tests failed to reveal the presence of alkaloids.

Sucrose was isolated and identified.

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A Phytochemical Study of *Larrea Divaricata* Cav. with Special Emphasis on its Yellow Pigments*

By Gertrude M. Horn† and Ole Gisvold‡

Larrea divaricata Cav. Fam. *Zygophyllaceae* is a shrub that is indigenous to some of the southwestern states, i.e., Texas, Nevada, New Mexico, Utah, Arizona, and Mexico. It is well recognized by the natives under the common name, creosote bush.

The plant and various preparations made from it have long been used medicinally by the Indian tribes. It was used for a variety of ailments, but usually for those requiring an antiseptic agent.

A preliminary phytochemical investigation (1) of *Larrea divaricata* revealed that the chief constituent responsible for the antiseptic action of various extracts was nordihydroguaiaretic acid. This phenolic compound has also been shown to possess marked antioxidant properties (2). Small amounts of two yellow pigments were also isolated (1) but were incompletely characterized.

One of the purposes of this investigation was to isolate larger quanties of the yellow pigments for more complete characterization studies. Also because nordihydroguaiaretic acid is such a marked antiseptic and antioxidant it was desirable to find (*a*) the location of the phenolic constituents, (*b*) the part of the plant which contained the largest amount of the phenolic constituent, (*c*) the per cent of the phenolic constituents and (*d*) the per cent of nordihydroguaiaretic acid.

EXPERIMENTAL

I. Fractional Extraction of the Ether Extract with Sodium Bicarbonate, Sodium Carbonate and Sodium Hydroxide.—The following experiment was a type procedure that effected a fractionation of the ether-soluble phenolic substances so that some of them could be purified more readily. Twenty-five hundred (2500) grams of No. 20 powdered drug were extracted with ether in a Soxhlet for a period of two hours. A dark green clear ethereal extract was obtained which was concentrated to a volume of 1500 cc. This extract was shaken with 60-cc. portions of 5 per cent solution of sodium bicarbonate. Ten to twelve 60-cc. portions of the bicarbonate solution were necessary to remove all the sodium bicarbonate soluble material. Upon acidulation, the bicarbonate soluble fraction yielded a small amount of a dark green amorphous material. The ether solution was next extracted with six 60-cc. portions of a 5 per cent solution of sodium carbonate containing $1^1/_2$ per cent of sodium hydrosulfite. Acidulation of the carbonate-soluble material yielded an amorphous dark orange-red material which was later shown to contain considerable amounts of the yellow pigments. The ether solution was lastly extracted with eighteen 60-cc. portions of a 5 per cent solution of sodium hydroxide containing $2^1/_2$ per cent sodium hydrosulfite. The following kinds of precipitates were obtained from the sodium hydroxide fractions upon acidulation:

(*a*) The first five portions yielded heavy yellow amorphous masses which rapidly turned almost black in color. The dark precipitate was covered with a layer of fine yellow-red material, very similar in appearance to that found in the sodium carbonate fractions.

(*b*) The sixth, seventh and eighth fractions, upon acidulation followed by warming on the steam bath, yielded yellow amorphous materials which were almost granular in consistency.

(*c*) The next eight fractions when treated in the same fashion yielded solid crystalline material with varying shades of yellow. These fractions gave a very characteristic color test for nordihydroguaiaretic acid with ferric chloride and sodium carbonate, as follows: Ferric chloride when added to a hydroalcoholic solution of nordihydroguaiaretic acid gives a green color that changes to lavender, blue, and finally wine-red when a dilute solution of sodium carbonate is added.

(*d*) The seventeenth fraction formed an emulsion which upon acidulation gave a blackish green oily mass.

(*e*) The last two fractions gave upon acidulation amorphous greenish precipitates which contained granular material.

II. Fractional Extraction of the Ether Extract with Sodium Hydroxide.—Three thousand grams of No. 20 powdered drug were extracted with ether as in the preceding experiment and concentrated to a volume of about 1500 cc. This ether extract was extracted with about twenty-nine 50-cc. portions of 5 per cent sodium hydroxide containing $2^1/_2$ per cent sodium hydrosulfite. Each alkaline extract was acidulated soon after it was separated from the ethereal extract and subsequently warmed on the steam bath to remove the small amount of ether present. The first three fractions gave amorphous precipitates that were very dark orange in color. These fractions were rich in yellow pigments which crystallized in the solid mass upon standing. The following four fractions were orange and resembled those obtained with sodium carbonate in the preceding experiment. These also showed yellow crystalline pigment material upon standing. The next three fractions gave dark orange gummy precipitates, mixed with orange crystalline-like material after they had stood for some time. The next three fractions yielded dark orange to brown colored precipi-

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tates that apparently did not contain the orange crystalline-like material. The ten succeeding fractions gave solid crystalline fractions that varied in their shades of yellow and gave a positive ferric chloride test for nordihydroguaiaretic acid. The last eight fractions contained much crystalline material that was apparently nordihydroguaiaretic acid together with some yellow pigments and other amorphous phenolic substances.

It was also shown that the preceding large number of fractions could be modified so that the pigments and amorphous phenolic substances could be first removed by several large extractions of 200 cc. each. These extractions could also be followed by several large extractions which gave quite a satisfactory preparation of nordihydroguaiaretic acid.

III. Ethylene Dichloride Extraction.—Thirteen hundred grams of a No. 20 powdered drug were exhaustively extracted in a Soxhlet apparatus with ethylene dichloride. The solvent when removed left a very dark green, almost black, gummy mass. This solid extract was dissolved in about a liter of ether and treated as described under I with much the same results. However, some of the sodium hydroxide fractions were contaminated with much green material.

IV. Alkali Extraction of the Powdered Drug.—Five hundred grams of the powdered drug were macerated for fifteen minutes with 1700 cc. of a 5 per cent solution of sodium hydroxide containing $1^1/_2$ per cent sodium hydrosulfite. The mass was strained through several layers of gauze. An aliquot of 850 cc. of alkaline extract was obtained which was acidulated with concentrated hydrochloric acid. A greenish yellow flocculent precipitate was obtained which after standing seventy-two hours assumed an orange-brown color. The supernatent liquid was decanted and the precipitate dissolved in ether with the aid of a little alcohol and acetic acid. The ether was washed with water and then concentrated until an ether-free residue was obtained. This residue weighed 15.15 Gm. or 6.06 per cent. The supernatant liquid was extracted with ether in a liquid-liquid extractor. One and six-tenths Gm. of solid material were obtained or 0.64 per cent.

V. Alkaline Extraction of the Unground Plant, Twigs and Leaves.—The general appearance of the crude plant, especially the exudation at the nodes, indicated that most of the phenolic material was on the surface of the plant and therefore could be obtained by direct extraction of the unground material. In the preceding experiments using the ground plant, a significant amount of desirable phenolic material was lost by absorption. This should be eliminated largely by the use of unground plant material.

Five hundred grams of small twigs and leaves were broken into small pieces and treated as described under IV. From this 59.88 Gm. of ether-soluble material, or 11.9 per cent, was obtained. This was almost double the per cent obtained under IV; however, the powdered material used in the former experiment contained the large stems which did not appear to have much resinous exudate on the surface.

In order to determine whether or not this alkaline procedure would remove most of the phenolic fraction, the marc from the above procedure was treated as follows. It was washed several times with water, followed by a washing with very dilute acetic acid, and then washed with distilled water to remove all the acid. The marc was then dried and ground to a No. 20 powder. This powder was extracted with ether in a Soxhlet type continuous extractor. The ether solution was extracted with 5 per cent sodium hydroxide containing $2^1/_2$ per cent sodium hydrosulfite. Little, if any, alkali-soluble phenolic material was obtained.

VI. Alkali Extraction of the Unground Plant; Heavy Stems and Branches.—Fifteen hundred grams of the heavy twigs and stems were chopped into small pieces of about 1 to $1^1/_2$ inches in length. These were treated as in experiment IV. Fifteen and two-tenths Gm. of ether-soluble material which represents 1 per cent was obtained.

The alkaline-extracted woody material was washed with water followed by several washings with very dilute acetic acid, finally with distilled water. The woody material was then dried and ground to a No. 20 powder. This powder was extracted with ether in a Soxhlet type continuous extractor. The ether solution was extracted with 5 per cent sodium hydroxide containing $2^1/_2$ per cent sodium hydrosulfite. Little, if any, alkali-soluble material was obtained. These experiments indicate that little, if any, phenolic material is in the wood or bark of the larger stems.

VII. Alkali Extraction of the Unground Plant Followed by Fractionation of the Ether-Soluble Phenolic Material.—Twenty-five hundred grams of the unground drug consisting of the smaller stems, twigs and leaves were treated essentially as described under IV. However, the supernatant liquid was not subjected to a liquid-liquid extraction process but was mixed with "Filter-Aid" and filtered by suction. The phenolic fraction of the solid material thus obtained was dissolved in ether by the aid of a little alcohol and acetic acid as previously described. About a liter and a half of an ether solution was obtained which was dark red-brown in color. Fractionation of this ether extract as described under I yielded fractions that were quite similar. Essentially no material was soluble in bicarbonate. The first carbonate fractions were dark orange and the latter bright orange. The first alkali fractions were yellow-orange and the latter were light yellow. The fractions rich in nordihydroguaiaretic acid readily yielded 98 Gm. of solid crystalline material or 3.92 per cent. The last alkali fractions contained an oily phenolic substance. The ether solution that had been completely extracted with alkali was still pale yellow in color. It still contained a small amount of neutral pigment material.

Yellow Pigment

Isolation of "Yellow Pigment."—The "yellow pigment" was obtained from the sodium carbonate and first sodium hydroxide fractions. See I and II of the original ether or alkali extractions of the ground plant or VII of the unground plant. The sodium carbonate soluble fraction was treated with boiling 85 to 90 per cent alcohol. Crystalline, yellow pigments separated upon cooling. Fractional crystallization yielded deep orange-brown to fairly light yellow crystalline material. Repeated crystallization of the lighter yellow fractions yielded a product that constantly melted at 217°–218° C. More of this material could be prepared by fractional crystallization of the darker fractions.

The semisolid material obtained from the first sodium hydroxide fractions when allowed to stand for a week or longer showed the presence of crystalline yellow material. This semisolid material was mixed with cold methyl alcohol. The noncrystalline portion readily dissolved and the crystalline yellow pigments could easily be separated by filtration. Several washings with methyl alcohol gave good yields of material that could readily be recrystallized from 85 to 90 per cent alcohol. It also melted at 217°–218° C.

This yellow pigment was obtained as needles that were bright but light yellow in color. It is

soluble in alcohol, acetone and ether, and slightly soluble in chloroform and petroleum ether.

The following qualitative tests were applied:

Ferric chloride added to a 50 per cent alcoholic solution gave a deep yellow color that did not change upon the addition of dilute sodium carbonate.

A few crystals dissolved in glacial acetic acid gave a pale yellow solution which turned dark yellow-orange upon the addition of a few drops of concentrated sulfuric acid.

A few crystals gave a dark yellow-orange solution when dissolved in concentrated sulfuric acid.

Substitution of concentrated hydrochloric acid for sulfuric acid gave a deep lemon-yellow solution.

The compound dissolved in sodium hydroxide test solution to give a deep orange, almost red solution. Acidulation discharged the deep color and a yellow precipitate formed.

A negative Molish Reaction was obtained.

A positive reduction reaction (3) with magnesium and hydrochloric acid, as characterized by a wine-red color, was obtained. This indicated the presence of either a flavanone or flavonol type structure.

About 10 mg. were subjected to reduction (4) with sodium amalgam in ethyl alcohol. After acidulation with concentrated hydrochloric acid, the solution became reddish orange in color and when diluted with water a pale red precipitate formed very slowly. This indicated the possibility of either a flavanone, flavone or flavonol type of compound. However, flavones are not reducible by magnesium and hydrochloric acid. Thus, the pigment is either a flavanone or a flavonol.

A positive Wilson's boric acid test (5) was obtained. Pigments of the flavone group that have an alpha-beta unsaturated carbonyl group and a suitable peri auxochromic group, such as a hydroxyl group, give a positive Wilson's boric acid test. Flavanones do not give this test. Therefore, this pigment is either a flavonol or isoflavonol type.

Anal.—Calcd. for $C_{17}H_{14}O_7$: C, 61.81; H, 4.27. Found: C, 59.30; H, 4.51.

Methoxyl.—Calcd. for $C_{15}H_5O_5(OCH_3)_3$: 18.8. Found: 19.01, 19.03, 19.10.

Derivatives

Acetyl Derivative.—A crystalline acetate was readily prepared either by hot acetic anhydride and sodium acetate or in the cold with pyridine and acetic anhydride (6). The crude acetyl derivative was recrystallized several times from 90 per cent alcohol to give white needles that melted at 214°–215° C.

Anal.—Calcd. for $C_{23}H_{20}O_{10}$: C, 60.5; H, 4.38. Found: C, 59.67; H, 4.24.

Acetyl value.—Calcd. for $C_{15}H_3O_5(OCH_3)_2(Ac)_3$: 28.3. Found: 30.45; 30.36.

A modification of the procedure for determining acyl groups as given by Niederl and Niederl (7) was used. Approximately 30-mg. samples of the acetate were placed in a small reaction flask fitted with a dropping funnel, and a side arm containing a small removable condenser; the side arm in turn was connected to a small vertical condenser from which the distillate was collected. Four cubic centimeters of 1 *N* sodium hydroxide solution was added to the sample. The flask was immersed in a beaker of water, which was brought to boiling and kept at that temperature for one-half hour. The reaction mixture was then allowed to cool to room temperature, and the side arm condenser was removed after first being rinsed with a small volume of distilled water. The condenser was replaced with a rubber stopper. Four cubic centimeters of diluted sulfuric acid was added from a dropping funnel and then several cc. of water. A small boiling chip was added and distillation effected at such a rate that 1 cc. per minute was collected in the receiving flash. From time to time more water was added to the reaction mixture from the dropping funnel. The distillation was continued until about 50 cc. of distillate had been collected. To this distillate a few milligrams of barium chloride were added and the solution then quickly heated to boiling. No turbidity occurred. The hot solution was titrated with a standardized sodium hydroxide solution using phenolphthalein as an indicator. During the time the first sample was being titrated, another 10 cc. of distillate was collected. This was treated in the same manner as the first. The second sample was collected to make certain that all the acetic acid had been recovered by the distillation.

Methylation of the Yellow Pigment with Dimethyl Sulfate and Alkali.—One gram of the yellow pigment was methylated with dimethyl sulfate and potassium hydroxide in acetone according to Wolfrom (8). This methylation is carried out at the boiling point of acetone. The crude methylated product was very soluble in hot alcohol and upon cooling, very pale yellow crystals separated which upon microscopical examination appeared to be a mixture of two types of crystals. The crystalline material was treated with hot water which effected a separation of water-soluble and water-insoluble portions. The water-insoluble portion was oily while warm but solidified upon cooling. The hot water soluble portion separated on cooling in the form of very fine feathery yellow needles. Microscopic examination indicated these to be uniform. They melted at 152°–153° C.

Anal.—Calcd. for methoxyl, $C_{15}H_5O_2(OCH_3)_5$: 41.6; mol. wt. (Rast), 372. Found: 42.4, 43.2, av. 42.8; mol. wt. (Rast), 382.

Methylation of the Yellow Pigment with Diazomethane.—Four-tenths of a gram of the "yellow pigment" dissolved in about 40 cc. of acetone and cooled to 5° C. was mixed with an excess of diazomethane in ether. The reaction mixture was then allowed to warm up to 25° C. and the solvent evaporated spontaneously. The yellow crystalline material that formed was recrystallized from dilute alcohol; however, the resulting crystalline material also was a mixture. (See above methylation.) It was purified by means of hot water from which pale yellow, fine silky needles separated on cooling, m. p. 134°–136° C.

This methylated compound in 50 per cent alcohol gave an olive-green color with ferric chloride.

Demethylation of the "Yellow Pigment."—One-tenth of a gram of the yellow pigment, 2.5 cc. of hydriodic acid, sp. gr. 1.7, and 2.5 cc. of acetic anhydride were heated by an oil bath (130°–135° C. for one hour. The reaction mixture was then cooled to 25° C. and poured into a mixture of ice and water. The demethylated product separated as a pale green flocculent material. The product was recrystallized three times from dilute alcohol. The resulting yellow-green lustrous crystalline compound melted at 290° C.

Acetyl Derivative of the Demethylated Compound.—Five-hundredths of a gram of the above demethylated compound was acetylated by refluxing for two hours with acetic anhydride and sodium acetate. The crude acetyl derivative first separated as an oil, which became solid upon standing in the icebox overnight. When recrystallized several times from dilute alcohol, white needle-shaped crystals that melted at 184°–185° C. were obtained.

Orange Pigment

Isolation of Orange Pigment.—During the purification of the "yellow pigment" by fractional

crystallization, the last fractions were bright orange in color. These bright orange fractions on repeated crystallizations yielded a product that melted at 230°–232° C. with decomposition. The amount of "orange pigment" obtained was very small in comparison to the amount of "yellow pigment" obtained. It is soluble in alcohol, acetone, ether and chloroform, and slightly soluble in petroleum benzin.

The qualitative tests that were applied to the "yellow pigment" were also applied to the "orange pigment." The results were the same except that concentrated hydrochloric acid gave a pale orange-colored solution.

Qualitative analysis for elements indicated the absence of sulfur, nitrogen and halogen.

Anal.—Calcd. for $C_{15}H_{16}O_8$: C, 60.00; H, 4.44. Found: 61.05, 60.45; H, 4.80, 4.58. Calcd. for methoxyl, $C_{15}H_7O_5(OCH_3)_2$: 25.8. Found: 24.03, 24.19.

Derivatives:

Acetyl Derivative.—This pigment was acetylated by the same methods used for the acetylation of the "yellow pigment." After several crystallizations from alcohol, a white needle-shaped crystalline compound was obtained that melted at 222° C.

Anal.—Calcd. for $C_{15}H_4O_5(Ac)_2(OCH_3)_3$: C, 59.2; H, 4.52. Found: C, 59.48; H, 4.54.

Acetyl value: Calcd. for $C_{15}H_4O_5(Ac)_2(OCH_3)_3$: 26.54. Found: 27.71, 28.10.

Methylated Derivatives.—The "orange pigment" was methylated with dimethyl sulfate and alkali as described for methylation of the "yellow pigment."

Recrystallization from hot water yielded pale yellow needles that melted at 142–143° C.

Anal.—Calcd. for methoxyl, $C_{15}H_5O_3(OCH_3)_5$: 39.95; mol. wt. (Rast), 388. Calcd. for $C_{15}H_4O_2(OCH_3)_6$: 46.20; mol. wt., 402. Found: methoxyl, 43.28, 43.73; mol. wt., 404.

Demethylation of the "Orange Pigment."—Demethylation was carried out as described for the "yellow pigment." Upon several recrystallizations from alcohol a light green substance was obtained which melted at 284° C.

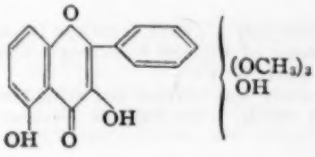
Acetyl Derivative of the Demethylated Compound.—Five-hundredths of a gram was acetylated with hot acetic anhydride and sodium acetate. The acetylated product was recrystallized several times from alcohol to give white needle-shaped crystals that sintered at 186°–188° C. and melted at 205°–208° C.

SUMMARY AND CONCLUSIONS

It has been shown in the experimental work that most, if not all, of the phenolic material of *Larrea divaricata* is present as a resinous exudate on the surface of the leaves and stems and that this phenolic material can be readily extracted with ether or aqueous sodium hydroxide containing sodium hydrosulfite to prevent oxidation. Successive extraction of an ether solution of the resin by sodium bicarbonate, sodium carbonate plus sodium hydrosulfite and sodium hydroxide containing sodium hydrosulfite effected a fractionation of the phenolic components. The sodium carbonate fraction was rich in pigments which could be readily purified to yield a yellow and an orange pigment. The latter sodium hydroxide extractions yielded almost pure nordihydroguaiaretic acid and furnished a practical method of preparation of the same. An amorphous phenolic fraction was obtained which comprised about 50 per cent of the resinous material.

The yellow and the orange pigments were subjected to partial characterization. Qualitative tests placed both pigments in the class of flavonols or iso-flavonols. Quantitative analytical data on the original "yellow pigment" and its acetyl and methylated compound best agreed with the following formulas: $C_{15}H_5O_5(OCH_3)_2$; $C_{15}H_5O_5(OCH_3)_2(Ac)_3$; and $C_{15}H_5O_2(OCH_3)_5$. Demethylation of the "yellow pigment" yielded a product that melted at 290° C. This corresponds very closely to that of the known flavonol morin (3,5,7,2′,4′-pentahydroxyflavone). Morin pentamethylether melts at 154°–157° C. and the completely methylated "yellow pigment" melts at 152°–153° C. The tetramethylether of morin (3,7,2′,4′ tetra methoxy 5-hydroxy flavone) melts at 131°–132° C., whereas the methylation of the "yellow pigment" with diazomethane yields a product that melted at 134°–136° C. The hydroxyl group in position 5 in these type compounds is not methylated by diazomethane. Further support of the presence of an hydroxyl group in position 5 is gained by an olive-green color with ferric chloride of the methylated compound that melted at 134°–136° C. It would appear that the "yellow pigment" is a dimethoxyl morin where the methoxyl groups are confined to the possible positions 3, 7, 2′ and 4′.

Quantitative analytical data on the original "orange pigment," its acetyl and methylated derivative best agreed with the following formulas: $C_{15}H_5O_3(OCH_3)_3$; $C_{15}H_4O_5(OCH_3)_3(Ac)_3$; and $C_{15}H_5O_3(OCH_3)_5$ or $C_{15}H_4O_2(OCH_3)_6$. Demethylation of the "orange pigment" yielded a product that melted at 284° C. A survey of the literature on flavonols revealed none that melted at the temperature of the original "orange pigment," its derivative or the demethylated product. From the data thus far reported it would appear that the "orange pigment" is a flavonol with a free hydroxyl group in position 5, having three methoxyl groups, and three acetylatable or methylatable hydroxyl groups. The following tentative formula in part can then be postulated:



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On the Toxicity of Hydroxyacetic Acid After Prolonged Administration: Comparison With Its Sodium Salt and Citric and Tartaric Acids*,†

By Stephen Krop and Harry Gold, with the technical assistance of C. Adelaide Paterno

The pharmacology of sodium hydroxyacetate was investigated by Riker and Gold in 1942 (1). It was shown that this compound is nontoxic in oral doses of 100 mg. per Kg., but that toxic effects resulted from doses of 250 mg. per Kg., which increased in severity as the doses were increased, doses of 500 mg. per Kg. proving fatal. It was relatively innocuous to the heart and circulation. The toxic effects involved gastrointestinal symptoms, neuromuscular disturbances, marked weakness and convulsions. A nephrotoxic action was discovered, resulting in tubular degeneration and marked azotemia. This study indicated that sodium hydroxyacetate is fairly completely absorbed from the gastrointestinal tract, that there is a fairly long latent period before symptoms appear even after intravenous injection, that the elimination of a nontoxic dose (measured as persistence of effect) is substantially complete in twenty-four hours or less, and that after toxic doses recovery may be delayed for several days, or death may occur after a period of several days.

This study was confined for the most part to observations on the cat and to acute experiments. There were experiments with large doses in two dogs, which indicated that the substance is also toxic to the dog. The three chronic experiments in the cat revealed the nephrotoxic action, but the observations were too limited to provide a satisfactory account of the possibilities of chronic poisoning by sodium hydroxyacetate.

The present study was planned to explore the effects of prolonged administration of hydroxyacetic acid, to compare its effects with those of sodium hydroxyacetate in the cat, and further to compare the response of the cat and the dog under similar conditions, since species differences in the response to organic acids are known to exist (2). Experiments were also included to ascertain the relative toxicity of hydroxyacetic, tartaric and citric acids in the dog.

EXPERIMENTAL

This report deals with the results of experiments successfully completed with 29 cats and 22 dogs. The drugs were given by mouth in a gelatin capsule, usually in a single dose. In the case of the doses of 500 mg. per Kg. or more, they were given in two fractions about six hours apart. In the first few experiments the drug was administered on an empty stomach. The local irritant action caused vomiting in thirty minutes or less. This difficulty was overcome by administering the capsule directly after a small quantity of milk or chopped meat.

The animals selected for the study were kept in the laboratory for a period of three weeks before the experiments were started in order to make certain of their good health.

The diet was fairly constant. The dogs received a dry kennel ration ("Red Heart" Diet B), approximately 120 Gm. daily made into a paste with water, in addition to an unlimited amount of dog pellets ("Big Red") and water. The cats received an unlimited amount of the above paste and water, in addition to 150 Gm. of meat daily. In the early experiments the dogs received 100 cc. of milk and the cats 50 cc. (condensed milk diluted with an

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TABLE I.—EFFECTS OF REPEATED ORAL DOSES OF HYDROXYACETIC ACID AND ITS SODIUM SALT IN CATS

			Blood Changes				Urine Changes					Outcome	
Cat No.	No. of Doses	Period of Administration, Days	Onset, Days	NPN[a], Mg. %	Creatinine,[a] Mg. %	CO_2[b], Vols. %	Onset, Days	Albumen	PSP[b]	RBC	Onset of Symptoms, Days	S or D[c]	Days
Sodium Hydroxyacetate, 250 mg./Kg.													
1	22	25	10	46–63	1.2–2.0	52–42	32	3+	0	0	15	D	32
2	12	12	10	43–136	1.2–7.5	–	10	±	0	0	10	D	13
3	45	50	10	50–470	1.4–17.0	51–31	15	1+	45–0	+	18	D	57
4	18	18	10	48–350	1.4–10.8	43–37	14	–	63–16	+	15	D	25
5	18	21	7	51–170	1.3–5.8	44–30	24	–	40–0	+	5	D	27
6	36	40	10	48–232	1.4–7.2	39–32	48	0	45–0	0	8	D	49
Hydroxyacetic Acid, 194 mg./Kg.													
1*a*	16	18	10	56–109	1.0–5.1	44–35	–	0	–	0	17	D	22
2*a*	9	9	–	–	–	–	–	–	–	–	8	D	10
3*a*	44	49	16	46–228	1.8–6.9	46–30	35	0	50–0	+	16	D	56
4*a*	15	18	9	70–136	1.7–3.8	45–24	19	0	40–0	0	12	D	21
5*a*	22	26	7	47–102	1.3–3.2	52–21	28	0	48–0	0	12	D[d]	29
6*a*	9	10	7	41–125	1.1–3.9	–	7	±	–	0	9	D	10
7*a*	12	13	7	49–194	1.3–4.4	48–35	–	0	–	0	8	D[d]	21
8*a*	5	7	7	47–185	1.3–7.0	–	–	0	–	0	4	D	11

[a] The NPN and creatinine changes were usually progressive; the first figure shown is the highest of the controls and the second figure the highest during the use of the drug.
[b] The changes were progressive; the first figure is the lowest of the controls and the second the lowest during the use of the drug.
[c] Survived or died.
[d] Polyuria and polydipsia in late stages of poisoning.

TABLE II.—EFFECTS OF REPEATED ORAL DOSES OF HYDROXYACETIC ACID AND ITS SODIUM SALT IN CATS

			Blood Changes				Urine Changes					Outcome	
Cat No.	No. of Doses	Period of Administration, Days	Onset, Days	NPN[a], Mg. %	Creatinine[a], Mg. %	CO_2[b], Vols. %	Onset Days	Albumen	PSP[b]	RBC	Onset of Symptoms, Days	S or D[c]	Days
Sodium Hydroxyacetate, 125 mg./Kg.													
1	50	54	7	78–100	1.7–2.7	0	32	0	0	0	7[d]	S	86 (sacrificed)
2	50	53	21	62–81	0	0	15	2+	0	+	–	S	–
3	42	44	27	50–82	0	0	–	0	0	0	–	S	–
4	43	46	7	63–102	1.8–4.0	0	–	0	0	0	44	S	86 (sacrificed)
5	50	53	7	79–99	0	0	32	0	49–9	0	–	S	86 (sacrificed)
6	50	53	7	53–70	0	0	42	3+	0	0	–	S	–
Hydroxyacetic Acid, 97 mg./Kg.													
1*a*	55	59	14	52–65	0	47–34	20	1+	0	+	–	S	–
2*a*	36	39	7	52–85	1.3–1.8	0	–	0	0	0	7	S	–
3*a*	29	31	30	53–88	1.4–1.7	48–34	–	0	–	0	20	S	–
4*a*	26	28	7	78–245	2.2–10.0	40–30	20	1+	–	0	20	D	29
5*a*	43	46	7	54–108	1.4–2.5	0	32	0	64–26	+	20	S	48 (sacrificed)
6*a*	38	40	29	50–580	1.6–26.5	43–35	29	0	46–0	+	20	S	40 (moribund, sacrificed)

[a] The NPN and creatinine changes were usually progressive; the first figure shown is the highest of the controls and the second figure the highest during the use of the drug.
[b] The changes were progressive; the first figure is the lowest of the controls and the second the lowest during the use of the drug.
[c] Survived or died.
[d] Symptoms lasted six days and subsided.

equal volume of water) immediately before the dose of the drug. In the later experiments, approximately 100 Gm. of meat was substituted for the milk in the case of the dogs.

Observations.—The course of the experiments was followed by observation of the general appearance and behavior of the animals, the weekly weight, weekly determinations of the blood NPN and creatinine, and simultaneous examination of the urine for specific gravity, *p*H (glass electrode), albumen and blood cells. At intervals of three weeks, the serum CO_2 combining power and kidney function

by the PSP test were determined. In addition to this routine, some of the tests were carried out more frequently as indicated by the course of poisoning. There were several sets of values of the foregoing tests before and during the use of the drugs: 2 to 5 (average 3) before the drug; 1 to 14 (average 6) after the drug in the fatal cases; and 4 to 15 (average 9) in the survivors. The urine was obtained in cats by expression and in dogs by catheter. Some of the animals were sacrificed. In all cases gross postmortem examinations were made and, in some, sections of the liver, kidney and lung were prepared for histological examination.

TABLE III.—EFFECTS OF REPEATED ORAL DOSES OF SODIUM HYDROXYACETATE AND HYDROXYACETIC, TARTARIC AND CITRIC ACIDS IN DOGS

			Blood Changes				Urine Changes					Outcome	
Dog No.	No. of Doses	Period of Administration, Days	Onset, Days	NPN[a], Mg. %	Creatinine[a], Mg. %	CO_2[b] Vols. %	Onset, Days	Albumen	PSP[b]	Casts	Onset of Symptoms, Days	S or D[c]	Days
					Sodium Hydroxyacetate, 250 mg./Kg.								
1	35	49	—	0	0	0	—	0	0	0	—	S	49[d]
2	84	100	—	0	0	0	45	0	0	Occ. hyaline	—	S	100[d]
3	107	115	—	0	0	0	—	0	0	0	—	S	115 (sacrificed)
4	84	100	—	35–109	0	0	58	3+	0	0	—	S	100[e]
					Hydroxyacetic Acid, 194 mg./Kg.								
5	85	101	—	0	0	0	—	0	0	0	—	S	—
6	86	101	—	0	0	0	—	0	0	0	—	S	—
7	84	101	—	0	0	0	—	0	0	0	—	S	101 (sacrificed)
8	24	36	24	0	0	56–15	—	0	0	0	—	S	38[f]
					Hydroxyacetic Acid, 500 mg./Kg.								
9[g]	104	119	—	0	0	0	—	0	0	0	—	S	119 (sacrificed)
10	103	119	—	0	0	0	—	0	0	0	—	S	120 (sacrificed)
11	82	89	—	0	0	0	—	0	0	0	—	S	89[h]
12	104	119	—	0	0	0	—	0	0	0	—	S	120 (sacrificed)
					Citric Acid (U. S. P.), 1380 mg./Kg.								
13[i]	108	119	—	0	0	0	—	0	0	0	—	S	119 (sacrificed)
14	109	120	—	0	0	0	—	0	0	0	—	S	120 (sacrificed)
15	103	112	—	0	0	0	—	0	0	0	—	S	—
					Tartaric Acid (U. S. P.), 990 mg./Kg.								
16	77	90	88	36–116	1.1–3.4	0	38	1+	0	Hyaline and granular	45	D	90
17	100	114	—	0	0	0	—	0	0	0	—	S	120 (sacrificed)
18	103	114	—	0	0	0	89	0	0	Hyaline	—	S	120 (sacrificed)
19	103	112	—	0	0	0	19	0	0	Hyaline	—	S	—

[a] The NPN and creatinine changes were usually progressive; the first figure shown is the highest of the controls and the second figure the highest during the use of the drug.
[b] The changes were progressive; the first figure is the lowest of the controls and the second the lowest during the use of the drug.
[c] Survived or died.
[d] Died of injury by other dogs.
[e] Died of meningitis on 100th day when this NPN value was observed.
[f] Animal kept from food by others; seems to have died of starvation (weight loss, 39%).
[g] This animal was dog 5 in previous series.
[h] There were indications that the animal died of pneumonia.
[i] This animal was dog 6 in previous series.

Results.—The results of the experiments with cats are summarized in Tables I and II. Each of six cats (Table I) received a daily oral dose of 250 mg. of sodium hydroxyacetate per Kg. Symptoms (anorexia, weakness, depression, vomiting, loss of weight) began in from five to eighteen days. They progressed and terminated in coma and convulsions, with death in from 13 to 57 days. All animals lost weight, from 10 to 29%. Evidence of renal damage appeared in from 7 to 10 days. The blood showed advanced nitrogen retention. Albumen appeared in the urine, and the PSP excretion and the alkali reserve of the blood fell markedly. These are changes seen in advanced renal failure.

Each of 8 cats (Table I) received a daily oral dose of hydroxyacetic acid, 194 mg. per Kg., the molecular equivalent of the 250-mg. dose of the sodium salt. The experiments with the acid were in all cases conducted simultaneously with those in which the sodium salt was used. The toxic effects of the acid and the course of the poisoning were indistinguishable from those with the sodium salt at this dosage level. The weight loss in this group was similar and ranged from 9% to 30%.

In view of the foregoing toxic effects of the 250-mg. dose of sodium hydroxyacetate and of its molecular equivalent in the form of the acid, another series of

experiments was carried out in a similar manner, using a smaller dose of sodium hydroxyacetate, 125 mg. per Kg., and its molecular equivalent in the form of the acid, 97 mg. per Kg. The results are summarized in Table II. The 125-mg. dose of the salt rarely caused symptoms and was not fatal when administered daily for from 44 to 54 days in each of six cats. It was, however, nephrotoxic and produced azotemia. Nitrogen retention was probably present in all and it was fairly marked in some. These blood changes usually occurred in the absence of symptoms. The weight changes were small and varied from a gain of 13% to a loss of 8%; four of the cats gained weight. The changes in blood chemistry were, therefore, not the result of impairment of general nutrition.

A molecular equivalent dose, 97 mg. per Kg., in the form of hydroxyacetic acid given daily to each of six cats was distinctly more toxic, producing symptoms in from 7 to 20 days with much more marked urinary and blood changes. In this group there was one fatality. Four of the six animals lost weight (7 to 24%), one gained 8% and one remained unchanged.

Each of three cats was maintained without drug under the same conditions simultaneously with the treated animals for control purposes. They remained well, and all urine and blood analyses remained normal.

It may be noted that in the previous report (1) it was found that the 100-mg. dose of sodium hydroxyacetate may be given daily orally without toxic effects. This is apparently close to the largest dose which may be so given with impunity in view of the present findings that the 125-mg. dose exerts a nephrotoxic action in the cat.

The experiments with the dogs are summarized in Table III. Each of four dogs received a daily oral dose of 250 mg. per Kg. of sodium hydroxyacetate for periods of from 49 to 115 days. These produced no symptoms; the weight remained constant or increased; there was no evidence of renal injury, with one exception which showed an occasional hyaline cast during pregnancy, and another which showed albuminuria. The latter died of meningitis and developed a high blood NPN on the day of death. One dog (No. 2 of Table III) littered on the eighty-first day after having received 68 doses of 250 mg. of sodium hydroxyacetate. One puppy died at birth while the remaining two appeared normal. Sections of the kidney, liver and lung made of one of the survivors were histologically normal.

The results were similar for the four dogs which received a molecular equivalent dose of hydroxyacetic acid, 194 mg. per Kg. The dog, therefore, seemed to be more tolerant than the cat. The dose was then more than doubled and each of four dogs received 500 mg. of hydroxyacetic acid daily for periods of from 89 to 119 days. These also failed to produce any symptoms, weight loss, or urinary or blood changes. One of the dogs which received the 500-mg. dose for 82 doses had also previously received the 194-mg. dose for 85 doses.

These results indicate that the dog is at least five times as tolerant as the cat to sodium hydroxyacetate and hydroxyacetic acid by oral administration.

Each of three dogs received a daily oral dose of 1.38 Gm. of citric acid per Kg., the molecular equivalent of 0.5 Gm. of hydroxyacetic acid, for periods of 112 to 120 days. These produced no symptoms or evidence of renal damage.

Each of four dogs received a daily oral dose of 0.99 Gm. of tartaric acid per Kg., the molecular equivalent of 0.5 Gm. of hydroxyacetic acid, for periods of from 90 to 114 days. Casts appeared in the urine of three dogs; the blood chemistry remained normal except in one in which azotemia developed with death in ninety days. Weight changes varied from a gain of 30% to a loss of 32%. Others (2) have reported renal damage after similar oral doses of tartrate in dogs.

Histological Sections.—Sections of the liver, kidney and lung of each of three cats which had been poisoned with hydroxyacetate were examined histologically (cats 3 and 4*a* of Table I and cat 6*a* of Table II). These showed no changes in the lung, cloudy swelling with vacuolization and some central necrosis in the liver, but the most marked changes occurred in the kidney, namely, advanced tubular degeneration with relatively slight changes in the glomeruli. Similar sections were made in the case of one dog which had received hydroxyacetate (dog 11 of Table III), one dog with citrate (dog 13 of Table III), one dog with tartrate (dog 16 of Table III) and an untreated control dog. Similar sections were also made in the case of a puppy born of dog 2 of Table III, during treatment with hydroxyacetate. All tissues were normal with the exception of the kidney of the dog receiving tartrate. Examination of this kidney revealed fairly advanced tubular degeneration.

SUMMARY AND CONCLUSIONS

1. Hydroxyacetic acid and its sodium salt are nephrotoxic after oral administration in the cat.

2. The acid is more toxic than its sodium salt in the cat.

3. A dose of 100 mg. of sodium hydroxyacetate per Kg. is about as much as may be given orally daily without toxic effects in the cat.

4. The dog is at least five times as tolerant as the cat and shows no toxic effects from doses of hydroxyacetic acid as high as 500 mg. per Kg. daily for periods as long as about four months. The cause of the species difference in susceptibility requires a considerable amount of study.

5. A daily oral dose of 1.38 Gm. of citric acid, the molecular equivalent of the 500-mg. dose of hydroxyacetic acid, is without toxic effects in the dog, but an equivalent dose of tartaric acid, 0.99 Gm. per Kg., may be nephrotoxic.

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On the Influence of Several Anesthetics on the Fatal Dose of Digitalis in Cats and Frogs*,†

By Harald G. O. Holck, Edwin L. Smith‡, and Robert H. Shuler§

The present studies were started in 1937, but completion was delayed by several unforeseen interruptions, notably in animal supply. In this introduction we shall limit ourselves to selected comparisons of results in the standardization of digitalis and related cardiac glycosides with anesthesia by barbiturates or urethane as contrasted with ether; unless otherwise specified, cats were studied. Macdonald (1) secured values for digitalis 52 per cent higher with "liquid dial." With dial-urethane, Haskell (2) reported a lethal dose of digifolin 30 per cent greater and of digitalis 13 per cent higher. Bauer and Fromherz (3), in assaying ouabain, found a lethal dose 20 per cent higher with alurate (numal; isopropyl-allyl-barbiturate); with digitoxin alurate did not influence the results. In the studies by Vos and Dawson (4), it required about 25 per cent more digitalis with pentobarbital, and about 20 per cent more ouabain and scillonin. Kaplan and Visscher (5) in assaying lanatosides A, B and C obtained values from 41 to 48 per cent higher with pentobarbital; with lanatoside C the result was even 55 per cent greater in the dog with pentobarbital, which again was about the same as in unanesthetized dogs. Gruber, Haury and Drake (6), in dogs anesthetized exclusively with pentobarbital, noted values for digitalis 13 per cent above those with pentothal (thio-pentobarbital); the difference is probably significant. Fry and Swanson (7) reported results for digitalis 37 per cent higher with sodium amytal (isoamyl-ethyl-barbiturate) and 27 per cent greater with seconal (propyl-methyl-carbinyl allyl barbiturate). According to Haag and Woodley (8), fatal doses were slightly, but not significantly higher for digitalis with pentobarbital or amytal in pigeons. As to urethane, David and Rajamanickam (9) reported values for digitalis not significantly different from those with ether (5 cats only with each). On the other hand, Edmunds, Moyer and Shaw (10) secured values for this drug 20 per cent higher with urethane.

EXPERIMENTAL

The cats used in these studies were kept on a standard, adequate diet, including milk, in large airy cages in a well-ventilated room. They were kept a minimum of nine days before being used. The minimum weight was 1.7 Kg. and, except in case of pentobarbital anesthesia, in which we included extra heavy cats, the upper limit was 3.5 Kg.; these limitations are about those employed by David and Rajamanickam (9). All major groups (Table I) contained an equal number of male and female cats. The animals were deprived of food for a period from four to six hours before the assay, according to the plan of Eggleston (11).

Our tinctures were made from the U. S. P. Reference Standard Digitalis Powder (1936) according to the directions of this Pharmacopœia (12); hence the 745 mg. actually weighed out in the calculations are considered as 1000 mg. The final preparation was stored in an electric refrigerator and used within six weeks. The tincture was diluted 25 or 50 times with Locke's solution shortly before the perfusions were started. Just before each assay, the properly diluted tincture was placed in a 100-cc. burette provided with a jacket containing water warmed sufficiently to keep its contents at approximately body temperature. All assays were carried out on a specially constructed table, containing within it three separately controlled electric light bulbs and a small electric fan so that the temperature of the copper top could help maintain the body temperature of the animal. The tincture was injected into a femoral vein at the rate of 0.50 cc. per Kg. per minute, and this rate was carefully controlled from minute to minute. In some of the earlier experiments a tracheal cannula was introduced; however, this was found unnecessary because the cats apparently did not exhibit, with intramuscular administration, the laryngeal spasm reported by Burstein and Rovenstine (13) for the intravenous injection of pentobarbital and certain other barbiturates.

Ether was administered by the drop method on a muslin-covered wide mesh wire cone. The 5 per cent pentobarbital sodium solution was administered intramuscularly; 35 mg. per Kg. were found insufficient, hence we started with 40. Additional doses

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‡ Most of the ether and pentobarbital assays have been taken from a thesis submitted by Mr. Smith to the Graduate College in the University of Nebraska in partial fulfillment of the requirements for the degree of Master of Science.

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of 5 or of 2.5 mg. per Kg. were administered whenever the initial doses did not produce anesthesia. About three-fourths of the animals were satisfactorily anesthetized with 40 to 45 mg. per Kg.; the largest dose given to any cat was 55 mg. per Kg. The corneal reflex was maintained in all cases; unnecessarily large doses were carefully avoided because when the respiratory rate falls to below 30 per minute, the consequent anoxemia causes circulatory disturbances, according to Reme, Lerche and Kuckulies (14). To prevent precipitation of the pentobarbital, we kept our reserve supply in a syringe which prevented contact with the air.

In case of dial sodium we also used a 5 per cent solution, but the initial dose was 50 mg. per Kg., intramuscularly, with a maximum dose of 65 mg. per Kg. in one cat. With urethane we employed a 33.3 per cent solution; the initial dose was 1.0 Gm. per Kg., intraperitoneally administered, and supplemented as needed; the maximum needed by any one cat was 1.5 Gm. per Kg.

The same as ours. The values with pentobarbital anesthesia were 47 and 49 per cent higher for the two dilutions, respectively, than with ether, using the arithmetic means as our bases; such a difference is nearly the same as that found by Kaplan and Visscher (5) for the lanatosides. With the barbiturate dial the value was 51 per cent higher than with ether, or practically identical with the difference ascertained by Macdonald (1). There was no significant difference between the fatal doses obtained with pentobarbital and with dial, respectively; but either was very significantly greater than that found with urethane, which again was significantly greater than that seen with ether. The values obtained with the 4 per cent solution of the tincture of digitalis were but 2.5 and 4 per cent higher than those with the 2 per cent solution; although there was no significant difference between the two averages, either under pentobarbital or under ether anesthesia, in both cases the lower value was secured with the greater dilution.

TABLE I.—SUMMARY OF DIGITALIS ASSAYS UPON CATS[a]

Group Anesthesia	No. of Cats	Weight Range, Kg.	Per Cent Conc. of Tr. Digit.	Mean Fatal Dose, Mg./Kg. Arithmetic	Mean Fatal Dose, Mg./Kg. Geometric	Groups	Critical Ratio Geometric	Ratio of Geometric Means
A Pentobarbital	100	1.69–4.74	4	94.3 ± 1.2[b]	93.5 ± 1.2[c]	A *vs.* C	10.1[d]	1.47
B Pentobarbital	20	1.76–3.46	2	92.1 ± 3.0	91.2 ± 2.9	B *vs.* A	0.8	1.03
C Ether	20	1.72–3.34	4	64.3 ± 2.3	63.5 ± 2.3	C *vs.* D	0.9	1.05
						C *vs.* E	4.1	1.20
D Ether	30	1.76–3.36	2	61.7 ± 2.0	60.7 ± 2.0	D *vs.* B	9.0	1.50
E Urethane	40	1.76–3.32	4	77.4 ± 2.1	76.3 ± 2.0	E *vs.* A	7.1	1.23
F Dial	20[e]	1.80–3.46	4	97.0 ± 2.9	96.3 ± 2.9	F *vs.* C	8.8	1.52

[a] The cats were injected intravenously at a rate of 0.5 cc. per Kg. per minute with a suitably diluted tincture of digitalis until cardiac arrest occurred. Ten cubic centimeters of menstruum were used to extract 0.745 Gm. of the U. S. P. Reference Standard Digitalis Powder (1936); the mg. per Kg. represents the corrected value of 1.000 Gm. per 10 cc. The dilutions were made with Locke's mammalian solution.

[b] The standard error calculated from the formula $\sqrt{\frac{(\text{Sum of Deviations})^2}{N(N-1)}}$; N is the number of animals.

[c] The standard error calculated from the formula $2.3026(LD_{50})s_y$; s_y is the standard error of the mean log-dose.

[d] Difference between means divided by the standard error of the difference $\sqrt{e_1^2 + e_2^2}$, both in terms of the log-dose.

[e] Sixty per cent of these cats showed tremors or even convulsions; this tendency is markedly diminished when dial-urethane (Ciba) is substituted for the pure barbiturate. No difference was seen between the 10 cats without and with the intestinal parasites, nor among cats with varying degrees of intestinal parasite infestation.

Except in the one series in which we gave artificial respiration and recorded the blood pressure, the final arrest of the heart, our end point, was determined by the use of the stethoscope in the manner used by Van Wijngaarden (15) and others. Observations were continued for at least three minutes after the heart had finally stopped. After death all cats were examined for lung infection (16), albuminuria (17), pregnancy (15), or any obvious abnormality. No pregnant cats are included in these series.

Results and Discussion

A summary of the results of the cat assays has been prepared in Tables I and II. In addition to the logarithmic values, the arithmetic means are given in Table I for comparison with published data on the U. S. P. Reference Standard Digitalis Powder (1936). These results may be discussed under various headings. First of all, the average fatal dosage of 64.3 mg. of digitalis per Kg. with ether anesthesia checks very well with the value of 65.0 reported by Edmunds, *et al.* (10); likewise, the value of 77.4 mg. with urethane anesthesia agrees closely with their value of 77.9 mg. for 24 cats with an injection time of forty minutes, practically

TABLE II.—SUMMARY OF ANALYSIS OF DIGITALIS ASSAYS UPON CATS WITH PENTOBARBITAL ANESTHESIA AND RESULTS WITH PULMONARY COMPLICATIONS[a]

Group	No. of Cats	Weight Range, Kg.	Geometric Mean Fatal Dose, Mg./Kg.
Whole	100	1.69–4.74	93.5 ± 1.2
Extra heavy	20	3.63–4.74	94.6 ± 2.9
Heavy	20	2.58–3.58	92.8 ± 2.7
Medium	20	2.13–2.54	92.7 ± 2.8
Light	20	1.70–2.11	93.7 ± 2.1
Artificial respiration; blood pressure recorded[b]	20	1.69–3.38	93.7 ± 3.0
Pulmonary Disease[c]	14	1.86–3.25	58.5 ± 2.3

[a] Procedure and abbreviations are the same as given in the legend of Table I.

[b] Cardiac arrest in this group was determined by fall of blood pressure to the base line; in all other groups the arrest was determined by the auscultatory method. This phase of the project was aided by a grant from the Committee of Therapeutic Research, Council of Pharmacy and Chemistry, American Medical Association.

[c] During an epidemic of distemper all regular assays were stopped. The cats which apparently had recovered were given digitalis and the results are shown in this line of the table.

As to the weight of the cats, the arbitrary division in the pentobarbital series (Table II) of those not given artificial respiration into four groups of decreasing average weight shows clearly that there was no significant difference between any two of these groups, either as to mean or to standard error. Likewise, the group of 20 cats to which artificial respiration was administered and in which the end point was taken by means of the final fall of blood pressure, showed no significant difference from any of the other groups in the pentobarbital series. The artificial respiration series was added at the suggestion of the late Professor C. W. Edmunds after the three lighter weight groups had been completed.

TABLE III.—ANALYSIS OF VARIANCE FOR VARIATION BETWEEN CATS TESTED ON DIFFERENT DAYS COMPARED WITH THAT BETWEEN CATS TESTED ON THE SAME DAY

	Degrees of Freedom	Sum of Squares	Mean Square	F
Between days	72	10,831.0	150.43	1.22
Within days	27	3,319.2	122.93	..
Total	99	14,150.2	142.93	..

There was no consistent difference related to sex. With urethane anesthesia the average fatal dose was higher in case of the males, giving a "t" factor of 2.36; however, the difference between the two groups each of 20 cats was mainly due to a few unusually resistant males. Another reason for considering this difference as fortuitous is that with pentobarbital anesthesia and 2 per cent tincture of digitalis, with 10 of each sex, the females had the higher value. The probability for $t = 2.06$ was greater than but very near $P = 0.05$. Also, when successive groups of 6 cats of each sex were compared, only in one of the three double groups was there an appreciable difference in favor of the males with urethane anesthesia.

As to disease, the 14 cats that were recovering from distemper gave an average fatal dose with pentobarbital anesthesia that fell even below that for normal cats with ether anesthesia (Table II); the fact that these cats seemed quite normal in appearance when used indicates that it may be advantageous to examine the lungs of the cats immediately after death in digitalis assays, certainly so if there has been any recent distemper epidemic at the source of the cats or if a result is very low. These findings are in agreement with the observations by Wyckoff, *et al.*, on administration of digitalis to patients with pneumonia (18). As to urine tests for albumin, the 7 cats which showed albuminuria were all in the series which had had distemper; hence such a routine test would seem unnecessary for normal cats. As to intestinal parasites, 10 cats with dial anesthesia showed absence of parasites, the other 10 varying degrees of infestation, ranging from very light to heavy; inspection of the individual results show that apparently even those with heavy infestation were as resistant to digitalis as those entirely free from parasites.[1]

Inasmuch as our assays were conducted when animals were readily available, it happened that 50 of the cats in the large pentobarbital series were used at the rate of 1 a day, and the other 50 at the rate of 2 or 3 per day. To see whether or not this would appreciably influence the results, a further analysis was carried out by Dr. C. I. Bliss of the Connecticut Agricultural Experiment Station, to whom we are indebted for the computations in Table III.

The variability between days exceeded that within days, but not to a significant degree. Dr. Bliss comments as follows: "Analysis in arithmetic units indicates so small a difference between the two variences or mean squares that one would not expect them to differ significantly if computed in the preferred terms of log-dose."

TABLE IV.—EXPERIMENTS ON THE U. S. P. REFERENCE STANDARD TINCTURE OF DIGITALIS (1936) UPON UNANESTHETIZED FROGS AND ON FROGS ANESTHETIZED WITH ETHER AND WITH PENTOBARBITOL SODIUM, RESPECTIVELY[a]

Group	Cc. of Tincture Per Kg. of Frog	Systolic Standstill		
		Controls	Ether	Pentobarbital
First day				
1	3.00	5/36	17/36	...
2	4.20	20/36	21/36	...
3	5.88	8/10	9/10	...
Second day (male frogs exclusively)				
1	3.00	6/30	...	3/30
2	4.20	13/30	...	7/30
3	5.88	25/30	...	20/30

[a] The U. S. P. XI one-hour method was employed, except that the temperature of the water was 25° C. For details of anesthetic methods, see text.

As to uniformity of results, an inspection of the standard deviations for comparable groups fails to show any differences between the anesthetics under study. The cats on pentobarbital in Group A of Table I were divided into 16 successive sets, each of 6 cats. The arithmetic means of these sets varied from 86 to 100 mg. per Kg., three-fourths of them falling within the range of 92 and 98 mg. per Kg. The percentage standard error averaged 5.10 and in 75 per cent of the sets it fell below the 5.7 per cent required in the U. S. P. XII assay. With six such successive groups under urethane anesthesia, the average was 5.95 per cent, with four groups below the 5.7 limit. With five such successive groups under ether anesthesia (2 per cent tincture), the average was 6.53 per cent, with two groups falling within the 5.7 per cent allowance.

Experiments upon Frogs

Although the present studies deal mainly with establishing more firmly the difference in digitalis dosage under a few selected anesthetics and not with the mechanism of such difference, an attempt was made to establish how the fatal dosage with ether and pentobarbital, respectively, corresponded to that in unanesthetized animals. Frogs were selected for this purpose. The U. S. P. XI one-hour method was adopted with the following modifications: (*a*) a temperature of 25° C. was used instead of the prescribed 20° C.; (*b*) an anesthetic was used in some of the groups of frogs.

Ether, used as the anesthetic for some of the frogs,

[1] These cats were examined for intestinal parasites after death by Mr. Shuler who majored in this field.

was administered in the following manner: The frogs were placed in pans containing a 1-cm. layer of a 1 per cent (by volume) aqueous solution. Within less than thirty minutes the frogs became anesthetized (i.e., they would lie on their backs) and remained in this condition and alive for several hours; the solution containing ether was changed every thirty minutes. Preliminary tests with pentobarbital sodium showed that 70 mg. per Kg. was the surely fatal dose and that 45 produced anesthesia without causing any fatalities in three hours. An 0.45 per cent solution was used and injected into the dorsal lymph sac.

In a preliminary experiment upon three groups of 60 frogs each, with each group being divided into several subgroups, those frogs receiving digitalis thirty minutes after ether gave fatal doses slightly below—and those receiving it thirty minutes after pentobarbital, a little above the fatal doses of those that were unanesthetized. Hence a larger test was carried out and the results are presented in Table IV. Because of limited facilities, the tests had to be divided over a period of two days. Dosage increases were 40 per cent.

Inasmuch as only one group showed any obvious difference from the controls when ether was administered, the data were analyzed statistically to determine whether the combined differences were significant; a similar analysis was performed with the data from unanesthetized and pentobarbitalized frogs. Dr. C. I. Bliss, who has proposed a method for such cases (19), kindly did this analysis for us; the results are as follows:

"In comparison with the controls, ether had the effect of increasing the apparent potency of digitalis by 22.4 per cent and pentobarbital of decreasing it by 24.0 per cent, the log ratios of potency being $M = 0.088 \pm 0.037$ and -0.093 ± 0.037. Both differences are significant (P between 0.02 and 0.01). All observations agree with parallel dosage curves on the log-probit axes ($b = 5.79$), and with $\chi^2 = 6.29$ and $n = 7$ for the entire experiment."

Thus, pentobarbital to a measurable extent protected the frogs against digitalis, whereas the reverse was true for ether. Since these studies were completed, Kaplan and Visscher (5), as stated, secured data with dogs indicating that those under pentobarbital anesthesia gave results of about the same magnitude as with those not anesthetized; thus, pentobarbital did not protect dogs against lanatoside C as it did frogs against digitalis.

SUMMARY

1. The mean arithmetic fatal dose of digitalis was 47 per cent greater with pentobarbital anesthesia in cats than it was with ether. With double dilution of the tincture, the increase in dosage was 49 per cent. With the barbiturate dial as the anesthetic, the dosage was 51 per cent higher, and with urethane only 20 per cent greater than with ether anesthesia.

2. Within the weight range of 1.7 to 4.7 Kg., the mean fatal dose of digitalis remained the same, as seen in four subgroups anesthetized with pentobarbital and arranged according to weight. Likewise, artificial respiration did not alter the dose.

3. Cats with pulmonary complications gave results showing low resistance to digitalis.

4. Analysis of variance revealed a small but not significantly greater variation between the results secured with cats on different days in comparison with those obtained on the same day.

5. Studies upon frogs indicate that pentobarbital increased and that ether anesthesia lowered the fatal dose of digitalis.

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A Study of the Responses of Intact Canine Uteri Following Orally Administered Ergot Alkaloids*

By H. F. Schloemer, F. T. Maher, and E. H. Wirth

Of the numerous methods which have been suggested for the assay of ergot and its preparations, none can be said to be entirely satisfactory. This conclusion is based upon the following considerations:

A. The various alkaloids responsible for the activity of ergot differ in many important respects. These differences are so significant that assay methods must evaluate separately the activity of the ergonovine[1] and the ergotoxine-ergotamine types of alkaloid.

B. Chemical methods of assay fail to distinguish between the active alkaloids and their inactive isomers (10, 12). Chemical assays likewise fail to reflect the pharmacological differences in the responses of animal uteri to the two types of alkaloid contained in ergot (10, 12). The quantitatively greater action of ergonovine on uterine preparations has been reported by numerous investigators (1, 5–9).

C. A good biological assay should measure the total activity of a given sample on the uterus, and should further fractionate this total to indicate the separate influences of the ergonovine and the ergotoxine-ergotamine groups of alkaloids. The official Cock's Comb Assay, based upon vasoconstriction in the highly vascular tissue of the cock's comb, does not measure directly a uterine response. Further, it does not recognize the relatively weak vasoconstriction effected by ergonovine (1, 2, 3), and does not recognize differences in uterine response to ergonovine and to the water-insoluble alkaloids. These differences are not evaluated in the other methods of bioassay in common use (1, 5–9).

Due to the increasing use of ergonovine in clinical medicine, there is need for a method of evaluating the distribution of alkaloid between the ergonovine and the ergotoxine-ergotamine groups in crude ergots. We have sought, therefore, a means of separating these two types of activity in laboratory animals.

Experimental studies by Thompson (14), Davis and his associates (4), Swanson and Hargreaves (11) and Moir (5, 7) indicate that only ergonovine is well absorbed from the intact gastrointestinal tract and that activity of ergot preparations orally administered is due primarily if not entirely to their ergonovine content. We have considered the possibility that a biological separation of ergonovine activity from that due to ergotoxine and ergotamine could be accomplished in an intact gastrointestinal tract.

In this investigation, we have therefore sought to evaluate the feasibility of such biological separation, as a possible basis for the separate evaluation of the two types of activity inherent in ergot. If it can be shown that the effects of orally administered ergot preparations are due entirely to ergonovine, then a ready means is at hand for the evaluation of ergonovine content in crude ergots, and this principle may further serve as a basis for the separate assay of ergonovine and of ergotoxine-ergotamine alkaloids contained.

EXPERIMENTAL

Procedures and Methods.—For this preliminary study, we have employed female dogs, nonpregnant and free from gross abnormalities. Each dog was anesthetized by means of an intraperitoneal injection of 35 mg. of pentobarbital sodium per Kg. of body weight. A balloon, approximately 0.5 x 3 inches in size, was then passed into the body of the uterus and attached to a water manometer. This water manometer was then connected to a tambour and uterine contractions were recorded as a stylus tracing upon a slow-moving kymograph. The water level in the manometer was adjusted to a uniform height above the level of the uterus in each animal to provide a uniform distention pressure in each experiment.

Upon the installation of this recording apparatus, a "normal" tracing was recorded for fifteen minutes. Each of ten dogs so prepared then received orally 0.4 mg./Kg. of ergonovine maleate, administered by stomach tube; each of a second series of 10 dogs received orally 0.4 mg./Kg. of ergotamine tartrate in the same manner. Following administration, tracings were recorded for a period of four hours. In a preliminary group of dogs so prepared, intravenous or intramuscular injection of either ergonovine maleate or ergotamine tartrate produced marked uterine responses.

Tracings of uterine contraction following the oral administration of ergonovine or of ergotamine were then evaluated in comparison with: (*a*) control tracings, in which the behavior of the uterus was followed for four hours following the insertion of the

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[1] The ergonovine maleate used in these experiments was furnished by Eli Lilly and Company.

balloon, with no drug administration; (*b*) the types of uterine response noted following the parenteral administration of ergotamine and of ergonovine in the preliminary animals.

Our method is thus a modification of that described by Swanson and his associates (11). These workers inserted their balloons into the uterine horns, which procedure necessitated an abdominal incision, with manual localization of the balloon. In our modification, this surgical trauma is avoided, and a good recording of changes in uterine motility was obtained in each instance.

Our interpretations of uterine motility changes in these two series of dogs are recorded in Tables I and II. Attempts in preliminary dogs to correlate uterine responses to ergot alkaloids with their responses to test doses of histamine were not conclusive and are therefore not included in these series.

Description of Tables.—Time of onset of response was determined by measuring the time interval between the oral administration of the drug and the first appearance of increased uterine activity. This onset of activity appeared as small, ripple-like contractions, or as large, rhythmical waves, depending upon the previous presence or absence of a normal rhythm, respectively.

Uterine activity gradually increased, with progressive increase in frequency and amplitude of contraction waves, until the uterus was in a state of constant contraction and relaxation, particularly with ergonovine. With ergotamine, a more irregular series of contractions, with incomplete relaxation, was commonly observed. In each case, a maximum level of activity was attained. The time of onset of this maximum response and the duration of maximum effect were measured on the completed tracing by time-clock markings.

The intensity of response was judged subjectively by an inspection of the completed tracing, in comparison with normal tracings, and with other tracings in these series, and by comparing the character, frequency and amplitude of contraction waves.

TABLE I.—RESULTS FOLLOWING ORAL ADMINISTRATION OF ERGONOVINE MALEATE (0.4 MG./KG.)

Dog No.	Weight, Kg.[a]	Normal Rhythm	Time of Onset of Response, Min.[b]	Time of Maximum Response, Min.[c]	Duration of Maximum Response, Min.	Intensity of Response
1	10.1	Absent	3	23	60	Marked
2	15.0	Present	11	50	90	Marked
3	10.5	Present	12	90	70	Marked
4	9.8	Present	18	120	60	Marked
5	19.5	Absent	10	10	180	Weak
6	15.8	Present	10	10	100	Weak
7	10.5	Absent	12	23	180+	Marked
8	15.4	Absent	11	27	165+	Marked
9	10.5	Present	12½	60	120+	Marked
10	9.0	Absent	3½	50	135+	Marked

[a] Av. 12.6/Kg. [b] 10.3/min. [c] 46.3/min.

TABLE II.—RESULTS FOLLOWING ORAL ADMINISTRATION OF ERGOTAMINE TARTRATE (0.4 MG./KG.)

Dog No.	Weight, Kg.[a]	Normal Rhythm	Time of Onset of Response, Min.[b]	Time of Maximum Response, Min.[c]	Duration of Maximum Response, Min.	Intensity of Response
11	11.5	Absent	18	90	150+	Marked
12	17.0	Absent	28	75	120+	Marked
13	13.5	Absent	12	105	135+	Fair
14	13.1	Absent	10	10	240+	Weak
15	11.5	Absent	31	105	150+	Marked
16	10.8	Absent	29	60	150+	Fair
17	8.5	Present	16	16	210+	Marked
18	8.3	Present	31	31	210+	Weak
19	6.9	Present	75	75	60	Marked
20	21.3	Present	13	31	60	Weak

[a] Av. 12.24/Kg. [b] 25.7/min. [c] 59.8/min.

DISCUSSION

Sex histories of the dogs used in this study are not available. Our dogs were assigned at random, with some attempt to maintain a gross balance in size and general characteristics between the two groups. We are interested here primarily in the comparative absorption of the two types of ergot alkaloid as recorded in uterine response; obviously, finer studies, if indicated, must deal with animals of known sex history since the behavior of the uterus is conditioned by previous pregnancies.

The number of observations presented here do not justify statistical evaluation. Our findings are sufficiently clear, however, to permit a preliminary evaluation of the feasibility of applying differences in absorption from the gut to the separate assay of ergonovine in ergot.

It is obvious from the tables presented that uterine responses following the oral administration of ergonovine maleate differ significantly from responses noted following the similar administration of ergotamine tartrate. These differences are particularly to be noted in the average times of onset of response, and in the average times of onset of maximum response.

These differences are neither uniform nor sharply defined, and there is, furthermore, considerable overlapping in responses between the two series. Thus, the times of onset in the ergonovine series varied from three to eighteen minutes, while a range of ten to seventy-five minutes was recorded in the ergotamine series. In five of the ten dogs in the ergotamine series, onset of activity occurred at times well within the range of variation noted for the ergonovine series. A similar finding is evident on comparing the times of onset of maximum response in the two series. Generally speaking, the duration of maximum response was considerably greater in the ergotamine group, but here again, no constant differences can be seen in the records.

The rapid development of activity, and of maximum response after oral administration of ergotamine tartrate is an interesting finding. It is our impression from the literature that only ergonovine is well absorbed from the intact gut, and that the activity of orally administered preparations of ergot is due primarily if not entirely to their ergonovine content (4, 5, 11,14). Our results do not substantiate this impression. Since there is considerable experimental evidence of antagonism between the two groups of ergot alkaloids (4, 12, 13, 14), the possibility of such antagonism occurring following the oral use of total ergot preparations must therefore be considered.

CONCLUSIONS

We have sought to evaluate the feasibility of employing selective differences in response to the several alkaloids of ergot, following oral administration, as the basis for a biological separation of ergonovine activity from that due to ergotamine. From this standpoint, our results justify the following conclusions:

1. Oral administration of ergonovine maleate is followed by a more rapid development of uterine stimulation than is noted following the oral administration of ergotamine tartrate. Differences noted were neither sufficiently distinct nor sufficiently uniform to validate the use of this phenomenon as a means of separating the activities of these two alkaloids in the intact female dog.

2. Our results, as indicated by the experimental evidence herein, do not substantiate the impression, gained from the literature, that the activity of orally administered ergot preparations is due predominantly to their ergonovine content.

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Book Review

Pharmacology, by MICHAEL G. MULINOS. Oxford University Press, New York, 1944. vi + 482 pp. 13.5 x 21.5 cm. Price, $4.00.

This book is one member of a "medical outline series." As the name implies, the information is presented in outline form but, with the use of completed sentences. For pharmacy students, and indeed also for medical students, the book cannot be used alone as the basic text. This does not detract from its value in reviewing pharmacology and in clinching knowledge learned previously in more detail. Although presented in outline form, there are very few omissions of the most fundamental information.

The limitations of this book are the expected failures of a book written in outline form. There is a natural tendency to discuss problems which are of varying shades of gray in pure black or white. For example, under the sympathomimetic drugs it is stated that the symptoms are "wakefulness, headache," etc. Wakefulness implies central stimulation and it has been clearly shown that only those sympathomimetic drugs with certain definite chemical structures exhibit such a phenomenon. At another point it is stated "the mode of action of chaulmoogra preparations in leprosy is uncertain, yet its value seems established." There is much antithetical evidence for such a bold statement.

There are a few errors in fact in this outline but most of them are of minor consequence. That it could be proper to establish a hypnotic dose for quinine is quite problematical. It is also difficult to justify the classification of acetanilid and acetophenetidin as sleep-maintaining hypnotics. On page 29 the statement found under "principle of oligodynamic action" is far from clear (probably due to its brevity). In considering the scope of pharmacodynamics it is doubtful whether this should include "the synthesis of the active constituent and the subsequent modification of the chemical structure in order to improve the therapeutic power of the compound."

The reviewer expects to have an ever-increasing need to refer to this book for assembling information about a given pharmacological class of drugs.—M. W. GREEN.